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ERRATA

Page 60, legend to Plate A, line 4, "tugidity" should read "turgidity."

Page 62, Table I, column 6, line 3, under "Per cent," "48.3" should read "4.83."

Page 72, line 14, "standards deviations" should read "standard deviations."

Page 137, Table II, column 4, line 3, "69" should read "59."

Page 207, paragraph 3, line 10, "the specimens" should read "other specimens."

Page 252, legend to Plate 33, D, "×90" should read "×1,000."

Page 340, line 30, "Murreaa" should read "Murrea."

Page 358, Plates 50–51, the plate numbers should be interchanged.

Pages 373–376 (Tables iv–xi) and pages 381–384 (Tables xiv–xxi), the headings for the abscissae and the ordinates should be interchanged.

Page 610, lines 9 and 10, "This was the only point at which *O. humilis* had been established when the collections were made" should read "At this point only *O. humilis* had been established when the collections were made."

Page 611, lines 15 and 28, "*Gibberella* spp." should read "*Gibberella* sp."

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# JOURNAL OF AGRICULTURAL RESEARCH

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TRUE NATURE OF SPINACH-BLIGHT[1] AND RELATION OF INSECTS TO ITS TRANSMISSION

By J. A. McCLINTOCK,[2] *Plant Pathologist, Virginia Truck Experiment Station*, and LOREN B. SMITH,[3] *Assistant State Entomologist, and Entomologist, Virginia Truck Experiment Station*

INTRODUCTION

For the past 10 or 15 years the greatest annual loss to the truck growers in eastern Virginia has been due to the trouble known as "spinach-blight." This was supposed to be a malnutrition disease caused by improper fertilization, and previous recommendations for its control comprehended the improvement of soil, fertilizer, and cultural conditions.

The shipments of spinach (*Spinacia oleracea*) from the eastern Virginia trucking region have averaged between 600,000 and 1,000,000 barrels annually, valued at $1,000,000 to $1,500,000. Owing to the ravages of the blight during the past two years (1916–17) the acreage has been materially decreased, and many growers have abandoned the crop entirely, while others grow it only on newly cleared land. Spinach is second in importance as a truck crop in this region, being surpassed in acreage and value only by the potato (*Solanum tuberosum*). Spinach is grown during the winter, and thus utilizes land which would otherwise be idle at that season.

The conditions which prevail and the losses sustained from spinach-blight have been carefully estimated for several years. From the data thus collected it appears that blight annually destroys or renders unfit for use not less than 20 per cent of the spinach crop. A conservative estimate of the annual money loss to the eastern Virginia growers by this disease is between $200,000 and $400,000.

The writers have found that this disease occurs in the spinach-growing regions of New York and Ohio under the name "spinach-yellows." Increasing losses may be expected in those States as the disease becomes

[1] The name "spinach-blight" is used throughout this paper because this is the name by which this disease has been known since its first appearance in Virginia.

[2] Collaborator, Office of Cotton, Truck, and Forage Crop Disease Investigations, United States Department of Agriculture.

[3] Detailed by the Virginia Crop Pest Commission for the investigation of insects affecting truck crops.

more prevalent. It is not unlikely that spinach-blight is present in other States where this crop is grown either for truck or seed purposes.

The majority of the experiments recorded in this paper have been performed during the past three years, and the direct inoculation experiments, under the controlled conditions of field and greenhouse cages, have been conducted since the fall of 1916. As the problem is far from complete and many important points are as yet unsettled this paper has been prepared as a preliminary report dealing with the nature of the disease, its dissemination, and transmission by insects.

In this cooperative undertaking certain of the generalized points herein presented have been proved by each worker independently, and the results then compared. The experiments relative to the insect transmission of the disease, together with the determination of the conditions by which this is influenced and controlled, were performed by the entomologist. The experiments on the relationship of soil, fertilizer, and seed conditions to spinach-blight, and the various virus inoculations to determine the points relative to the nature of the disease were performed by the plant pathologist.

DESCRIPTION OF SPINACH-BLIGHT

Spinach-blight is a specific disease characterized by mottling and malformation of the leaves (Pl. A). Although having many of the symptoms of the mosaic diseases of tobacco, cucumber, etc., spinach-blight differs from them in that the affected plants are eventually killed. (Pl. 1, A.) The disease appears either on plants scattered over the field (Pl. 1, B) or on many adjacent plants, thus forming a distinct area.

This blight may be distinguished from the various fungus diseases with which it may be associated, such as downy-mildew, caused by *Peronospora effusa* (Grev.) Rbh. (Pl. 2, A), Heterosporium leafspot, caused by *Heterosporium variabile* Cke. (Pl. 2, B), and anthracnose, caused by *Colletotrichum spinaciae* Ell. and Halst. (Pl. 3, A), because no microscopic organism is found associated with it. Spinach-blight may also be distinguished from diseases produced by the above-named fungi, by its causing a gradual degeneration of the tissues instead of definite leafspots. It has been observed that blighted plants are more susceptible to the attacks of certain fungi than are adjoining healthy plants. That the blighted plants are lower in vitality is shown by the fact that such plants are most seriously injured by occasional cold periods during the winter (Pl. 3, B).

Owing to the appearances of the blighted plants at various periods in the course of the disease, it is difficult to give a description which would be inclusive of their appearance at all times. For convenience eight purely arbitrary stages have been selected, as they offer rather marked

changes in appearance. The following is a description of the appearance of the plant at each stage of the disease:

STAGE 1.—Plants in this stage may be distinguished from the healthy plants by a very slight yellowing which generally occurs on the younger leaves or those which are not fully opened. Occasionally a slight yellowing may be noticed on one or more of the older leaves; otherwise the plant is in a vigorous growing condition and still retains a normal dark coloration (Pl. 4, A).

STAGE 2.—Plants in the second stage of the disease have a more pronounced yellowing on the younger leaves than those in stage 1. The plants are usually vigorous, and no marked changes are to be noted in the older leaves. Occasionally the younger leaves begin to show evidence of being malformed. This, however, is not usual in this stage.

STAGE 3.—This stage is characterized by the appearance of malformation in the younger leaves. They become much wrinkled and narrowed and show decided mottling. The yellowing has usually spread to many of the older leaves. Plants in the third stage of blight are generally not as large as healthy plants of the same age.

STAGE 4.—Plants in this stage show a distinct evidence of stunting; the yellow color has spread over the entire plant, and the older leaves are distinctly mottled. The younger leaves, while still growing to a certain extent, are so malformed as to be hardly recognizable as spinach leaves. They are very finely savoyed and have a feathery appearance. They do not curl to any great extent. The plant has lost most of its vigor by this time, and little growth takes place after this stage.

STAGE 5.—The fifth stage is characterized by the disintegration of tissues, usually shown by a browning and dead appearance of parts of the older leaves; the browning usually occurs on the outer tips first and works inward as the disease progresses. The younger leaves may become wholly yellow. This color is lighter in the fall and winter than it is in the spring, when it occasionally shows as deep orange-yellow. The mottled appearance of the older leaves at this stage is very striking; such chlorophyll as remains is gathered along the veins, leaving the tissues between the veins a pale-yellow color. The older leaves become wrinkled and lose much of the deep savoying characteristic of healthy leaves.

STAGE 6.—The sixth stage (Pl. 4, B) is characterized by the total disintegration of the older leaves; they become brown and lose turgidity, often being supported only at the point of attachment, the remaining portions resting almost entirely on the ground. About this time the central leaves of the plant begin to turn brown. The older leaves pass from a brownish yellow to a more or less translucent brown or straw color.

Stage 7.—The older leaves are practically disintegrated, with nothing but the petioles remaining. The younger leaves are brown, with no evidence of green and very little yellow color. The plant at this stage has reached a point of low vitality, but life still continues in the younger leaves and crowns. This is shown in Plate 5, B, *a*.

Stage 8.—The plant is dead, but has not entirely disintegrated (Pl. 5, B, *b*). At this stage a plant which was perhaps 15 inches in diameter before it was attacked by the disease is reduced to a small whorl of leaves scarcely an inch in diameter.

The root is apparently healthy and performs its normal function until the fourth stage is reached. From the fourth to the eighth stage the root gradually declines from the normal appearance. The root of a diseased plant is characterized by its shrunken appearance, a loss of lateral rootlets, and a browning of the internal tissues. This would indicate that the disease affects the foliage and aerial portions of the plant rather than the roots, the effect on the roots in all probability being secondary.

THE LENGTH OF THE VARIOUS STAGES

A more or less definite length of time elapses between the appearance of the first symptoms of the disease and the death of the host. In order to determine the length of the life cycle of the diseased plants under field conditions, the following experiment was performed.

Forty-one healthy and diseased plants were staked in the field on February 9, 1917. Records were made of the condition of the plants and the stage of progress of the disease. They were examined on February 22, March 3 and 22, April 2, 10, and 25. Each time records were made of the condition of each individual plant. The results of these observations are shown graphically in figure 1. The incubation period, or the time elapsing between inoculation and the appearanee of the first symptoms under field conditions, was determined from the inoculation experiments. The average time between the inoculation and the death of a spinach plant is 81.75 days, and the disease progresses gradually from the appearance of the first symptoms until the plant is dead. The slight variation shown in the length of the different stages is probably due to the variations in the time the observations were made. Theoretically the line representing the progress of the disease would be straight.

HISTORY OF SPINACH-BLIGHT

Spinach-blight was first observed at Lambert's Point, Norfolk County, Va., about 13 years ago and has since spread throughout the entire section.

Prior to 1907 spinach-blight had become so serious that spinach growers were asking for assistance in its control. The first experiments that were conducted with regard to the control of spinach diseases in Tidewater, Va., were undertaken by the Office of Cotton and Truck Dis-

ease Investigations of the United States Department of Agriculture. The first published reports on these experiments were issued as Bulletin No. 1 of the Virginia Truck Experiment Station in September, 1909.[1] From a discussion of the symptoms of the diseases studied at that time it would appear that the true spinach-blight was included. However, there were undoubtedly other diseases of truck crops which resembled spinach-blight in appearance, and the results obtained since that time would indicate that no distinction was made between them, all being classed as malnutrition diseases.

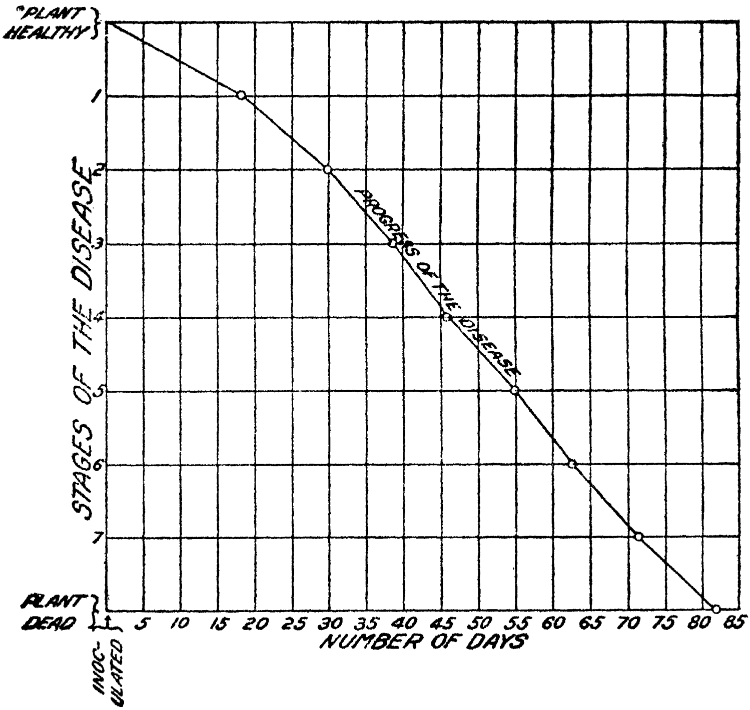


FIG. 1.—Graph representing the average lengths of the various stages in the progress of spinach-blight.

The work which was begun by Mr. L. L. Harter, of the Bureau of Plant Industry, was continued and published as Bulletin 4 of the Virginia Truck Experiment Station.[2] While this bulletin gave valuable suggestions for spinach culture, nevertheless it did not solve the spinach-blight problem. These data furnished the basis for distinguishing malnutrition diseases, which could be corrected by the use of proper fertilizers and lime, from the true spinach-blight, which subsequent experiments have shown has little or no relation to the fertilizer and lime content of the soil.

[1] HARTER, L. L. THE CONTROL OF MALNUTRITION DISEASES OF TRUCK CROPS. Va. Truck Exp. Sta. Bul. 1, 16 p., 4 fig. 1909.

[2] ——— SPINACH TROUBLES AT NORFOLK AND IMPROVEMENT OF TRUCKING SOILS. Va. Truck Exp. Sta. Bul. 4, p. 61–80, fig. 18–22. 1910.

CIRCUMSTANTIAL EVIDENCE OF THE NATURE OF THE DISEASE

For some years past growers have observed that spinach-blight followed within a reasonably short time after serious outbreaks of aphids. These observations pointed toward the possibility that aphids were the direct cause of blight. Recent experiments disprove this theory, and at the same time explain the indirect relation between the outbreaks of aphids and the subsequent epidemics of spinach-blight.

INOCULATION OF FIELD PLANTS WITH THE JUICE FROM BLIGHTED PLANTS IN THE WINTER OF 1915–16

In Bulletin 4 of the Virginia Truck Experiment Station,[1] Harter called attention to the fact that spinach-blight resembled the mosaic disease of tobacco, and plants in certain stages of spinach-blight have decidedly mottled leaves, quite characteristic of mosaic diseases. These observations, together with the indication that no microscopic organism appeared to be the cause of spinach-blight, led to the inoculation of healthy field plants with the juice of blighted plants. A bed of plants about one-half the size of marketable spinach was selected for this experiment. A quantity of blighted plants were collected on an adjoining farm, and the juice was obtained by mashing the tissues and straining through cloth. To serve as controls two rows of plants in the above-mentioned bed were pricked with a flamed needle, care being taken to wound both young and old leaves. The other rows of plants were inoculated by placing drops of juice from the diseased plants on leaves of various ages and pricking the juice into them with a flamed needle. These were kept under observation until they were large enough to harvest. No attempt was made to cover any of these plants, or to keep insects from them; but up to the time of inoculation no signs of blight had appeared in this bed or in any of the adjoining beds. Not long after the inoculations had been made, an outbreak of aphids occurred throughout the entire section. The plants on many farms became so badly infested that it injured their value for market purposes. Data collected from time to time showed that spinach-blight had developed on the inoculated plants. Blight also occurred to a limited extent on the control plants. This experiment gave merely an indication that spinach-blight is a communicable disease, and is spread in some manner, possibly by aphids, possibly by needle pricks when the juice is thus artificially carried, or possibly by some unknown agent.

RELATION OF DRAINAGE TO SPINACH-BLIGHT

It was the opinion of some growers that spinach-blight was directly associated with poor soil drainage; therefore the assistance of Dr. J. A. Bonsteel, of the Bureau of Soils of the United States Department of Agriculture, was obtained, and the relation of this factor to spinach-

[1] HARTER, L. L. OP. CIT., 1910.

blight determined as follows: Typical blighted areas in several fields were selected, and soil borings made to determine the texture and condition of both surface and subsoil. Similar borings were made in the same fields in areas where the plants appeared healthy. In no case was it found that the drainage was poor where the plants were blighted, and in some cases the subsoil in these areas was such that the drainage was much better within the blighted areas than in parts of the field where the plants appeared healthy. This would indicate that the matter of drainage has little or no direct bearing on the cause or spread of spinach-blight.

FERTILIZER EXPERIMENTS

RELATION OF FERTILIZERS TO THE CONTROL OF SPINACH-BLIGHT IN 1915

At the suggestion of Mr. L. L. Harter experiments were begun in which stable manure at the rates of 20 and 30 tons per acre and lime and gypsum at the rate of 1 ton to the acre were used in an attempt to determine the relation of these fertilizers to spinach-blight. Plots 0.1 acre in size were treated with the different substances, and similar untreated plots served as controls. The plots were planted with seed from the same lot and given similar cultural conditions. Spinach-blight developed as abundantly on the various treated plots as on the control plots; therefore it would appear that stable manure, lime, and gypsum have no influence on controlling spinach-blight.

SOIL AND FERTILIZER EXPERIMENTS ON SPINACH-BLIGHT CONTROL[1]

The relation of soils and fertilizers to spinach-blight was unsettled previous to 1915; therefore cooperative experiments were begun. A series of plot experiments were outlined which was to include various individual fertilizer elements and also combinations of fertilizers and complete fertilizers made up on acid and basic principles. Forty plots were used in this experiment. Blight was equally abundant on all of the plots. No data were obtained to indicate that any of the individual elements or combinations of elements had any direct relation to the control of spinach-blight.

Certain modifications of the above experiments were repeated in 1916. These substantiated the 1915 results, that fertilizers have no direct relation to spinach-blight.

RELATION OF FUNGI AND BACTERIA TO SPINACH-BLIGHT

Although previous workers had obtained results which indicated there was no specific fungus or bacterium which was the cause of spinach-blight, nevertheless it seemed advisable to repeat this work. Blighted spinach plants were collected from various fields and from different areas in the

[1] Conducted in cooperation with the Office of Drug-Plant, Poisonous-Plant, Physiological, and Fermentation Investigations, Bureau of Plant Industry, United States Department of Agriculture.

same field. Sections of diseased tissues from various parts of blighted plants were externally disinfected, and then plated on various types of nutrient media. The plates were incubated at various temperatures until organisms began to develop on the plates. Each type of organism was then transferred to agar slants, and grown in pure culture until its characteristics had developed. Both bacteria and fungi were isolated from various tissues of blighted plants. Plants in more advanced stages of blight yielded an abundance of organisms, while tissues from some of the plants in early stages of the disease remained sterile on the plates, thus indicating that no microscopic organisms were present in the tissues. Examination of the various cultures showed that some common organisms including species of Alternaria, Fusarium, Verticilium, Rhizoctonia, and Macrosporium were present, together with various of the lower soil fungi. Bacteria of numerous types and colors were also isolated from diseased tissues, but none of these appeared different from the common-soil and decayed-plant-infesting organisms. Subcultures of these organisms were prepared for inoculation work in the field, and with these a number of healthy field plants were inoculated by pricking the organism into the healthy tissues. A sufficient number of plants were used as controls. The inoculated plants and controls were under observation for a long time, but in no case did any of the inoculated plants consistently show a higher percentage of blight than the controls. These results led to the conclusion that none of the organisms which had been isolated in the laboratory from blighted plants was the specific cause of spinach-blight. The fact that the tissues from certain of the blighted plants, when externally disinfected and plated, remained sterile indicated that the disease could occur in a plant without any microscopic organism being associated with it. Therefore these results confirmed those of previous workers to the effect that spinach-blight is a definite disease, and is not due to any specific microscopic organism thus far obtained.

METHODS EMPLOYED IN THE SPINACH-BLIGHT EXPERIMENTS IN 1916–17

The cages used in the first experiments were furnished by the United States Department of Agriculture, but they were found to be too large for convenient work. The door was small, and the operator did not have room to reach conveniently to the corners of the cage. Therefore the cages used later[1] were smaller, the dimensions being 2½ by 3 by 2 feet; the frame was made of 1-inch material and the door occupied one whole side. This made an arrangement whereby the operator had access to all parts of the cage; yet the cloth was draped around him sufficiently to eliminate the possibility of the insects' gaining entrance through the large opening (Pl. 5, A). The door and manner in which it is made is shown in Plate

[1] The writers wish to acknowledge the suggestions of Mr. J. B. Norton, of the Office of Cotton, Truck and Forage-Crop Disease Investigations, Bureau of Plant Industry, in the development of the same.

6, A. The cloth used to cover the cages is known as light-weight sheeting. For aphid experiments it is necessary to use cloth with a close weave and yet not so close as to prevent a free circulation of air.

A type of small cage was developed which could be used to cover individual plants, or pots of plants on which insects were placed within the larger cages. These are shown in Plate 6, B. They consist of 12-mesh wire screen, rolled to a cylinder, and covered with a fine grade of sheeting or surgeon's gauze. Those with a diameter of 4, 6, or 10 inches were found most convenient. For use with spinach a convenient height is 12 inches, although for taller growing plants, as peppers and tomatoes, a height of 24 inches is preferable. The cloth is allowed to extend 7 or 8 inches beyond the top of the cylinder. It may then be drawn toward the center, gathered, and tied with a string, which permits the cylinder to be set over a pot and the plants examined through the opening made by loosening the string at the top. The cylinders were forced about 1 inch into the soil to prevent insects entering from below.

All individual plants or pots of plants within the larger field cages, on which aphids were placed, were covered with the small individual cages. At the end of the time the aphids were allowed to remain on experimental plants, the cages were fumigated with nicotine to kill the aphids. The plants were then carefully examined to insure that all the aphids had been killed and removed. Control plants were grown in each field cage, in order that the controls on each series might be under similar conditions of temperature, humidity, and soil. Records of conditions of temperature and humidity were made both within and without the field cages. The humidity was determined by means of wet and dry-bulb thermometers kept about 3 inches above the surface of the soil.

As no specific microscopic organism had been found associated with spinach-blight, the only means of proving infections was by further inoculations from the artificially infected plant, either by aphid transfers or by needle pricks. As certain forms of chlorosis (Pl. 10, B) and malnutrition, produced either by the lack of fertilizers or by the excessive applications of them, may cause the spinach plants to become similar in appearance to those affected with blight, the appearance and color of the plant could not be taken as positive evidence of the presence of the infectious disease. Two to five healthy plants were inoculated with the virus. When one or more of these plants developed a mottled appearance of the leaves characteristic of the disease, the original plant was credited with positive symptoms at the time the first color changes became visible in its leaves. Most of the secondary inoculations were performed in the greenhouse. As the season advanced and larger series, with higher percentages of infection occurred, only 5 to 50 per cent of the plants in each series were checked out in this manner. In the majority of cases the prick inoculations were made in the true leaves, but

in a few instances where the plants used were very young it was necessary to make the inoculations in the cotyledon leaves.

It was noticed in making the transfers of aphids to young spinach plants that they generally fed on the under side of the leaves rather than on the stems or petioles; hence, it is probable that in the majority of cases the inoculations were made from that point, when virus-bearing aphids were transferred to healthy spinach plants.

In making transfers or in any way handling the experimental plants the greatest care was exercised to keep the hands and instruments disinfected. Ninety-five per cent alcohol was used for this purpose. Great care was also exercised when making transfers of insects from diseased to healthy plants. The insects were carefully removed from a diseased plant by means of a camel's-hair brush, and were then placed on sterile glass slides on which they were carried to the healthy plants. They were removed from the slide by jarring it slightly. In this way neither instrument nor hand came in contact with the healthy plant. Needles and other instruments used in making prick inoculations were carefully flamed between each operation. In the field cages certain soil-inhabiting insects would occasionally gain access to the spinach plants and oftentimes attack them. This was almost entirely eliminated by thoroughly drenching the soil in the cage with a 1 to 100 solution of 40 per cent nicotine sulphate at the time it was set in the field.

In the majority of cases the precaution was taken to disinfect the spinach seed in 1 to 100 formaldehyde for a few minutes prior to planting. The majority of plants used in the greenhouse were grown from seed planted in steamed soil.

INSECTS COMMONLY ASSOCIATED WITH SPINACH AT THE TIME BLIGHT IS PREVALENT

Two species of Aphididae are primarily associated with spinach at the time blight is prevalent. These are the potato aphis (*Macrosiphum solanifolii* Ashmead) and the spinach aphis (*Rhopalosiphum persicae* Sulzer). Blight occurs most abundantly between the 1st of November and the 1st of April, and these aphids are prevalent at all times during the winter months. As will be shown, several other species of insects are capable of transmitting spinach-blight from diseased to healthy plants, but they are of minor importance in this connection. Thus far, these two species of aphids have been found to occur in Tidewater, Va., only as viviparous females. The true sexes have neither been collected nor reared. From our studies of the species it has been found that *M. solanifolii* is more active and reproduces more readily during periods of low temperature than *R. persicae*. Laboratory studies relative to these facts have been confirmed by field observations. The feeding habits of the two species are somewhat different. *M. solanifolii* are ungregarious, the alate and apterous forms move readily from plant to plant during

favorable weather, and may feed on many plants during their life. For this reason heavy infestations of this species within localized areas in a given field are rare. *R. persicae* has a tendency to congregate and, except during periodic outbreaks, are abundant only in more or less restricted areas within a field. As might be expected, the alate forms are the more active disseminators of spinach-blight.

Conditions which lead to a large production of alate females are indirectly responsible for the epidemics of blight which may follow. The production of alate females of *M. solanifolii* occurs throughout the winter months, and counts made at various times during the season showed that the ratio of alate to apterous forms was 4.12 to 1 for 2,500 individuals. Alate females of *R. persicae* are less numerous than the apterous form, except during a severe outbreak; under normal conditions the ratio of abundance of the two forms in the order mentioned is 1 to 47.41 for 2,500 individuals. Counts made at the time of an outbreak showed that the two forms were about equally abundant.

From its ability to withstand lower temperatures *Macrosiphum solanifolii* does not undergo the numerical fluctuations which occur with *Rhopalosiphum persicae*. The latter species occurs in great abundance, usually two or three times during the winter. Severe epidemics of spinach-blight often follow within a few weeks in the areas of heavy infestation.

FOOD PLANTS OF MACROSIPHUM SOLANIFOLII AND RHOPALOSIPHUM PERSICAE

Rhopalosiphum persicae attacks nearly all vegetable crops, as well as many weeds, shrubs, and trees. Over 60 species have been listed as its food plants, and undoubtedly there are others which are yet unknown.

Dr. Edith M. Patch,[1] in reporting her studies of *M. solanifolii*, gave a list of 20 plants which served as its food. In our studies of the species under southern conditions it has been found to be more cosmopolitan in its feeding habits than was hitherto supposed. The question of the relationship between the food plants of both *R. persicae* and *M. solanifolii* to spinach-blight has entailed a great amount of work which is as yet unfinished; hence, aside from mentioning that spinach is the most important fall and winter food plant of both species in this region, the findings in this connection are reserved for a later publication.

DIRECT INOCULATIONS WITH THE JUICE FROM BLIGHTED PLANTS

VIRUS INOCULATIONS

Blighted plants were collected from a field of young spinach and brought to the laboratory, where they were mashed in a mortar and the juice strained through a cloth. With a flamed needle the first row of

[1] Patch, Edith M. Pink and green aphid of potato. Maine Agr. Exp. Sta. Bul. 242, p. 205–223, fig. 47–49. 1915.

plants in a field cage was pricked, and the juice from the blighted plants then sprayed on them with an atomizer. The wet leaves were again pricked with a flamed needle to insure the entrance of the virus into the tissues. An adjoining row of plants in the same cage was pricked with a flamed needle to serve as controls. Sixty-two days after inoculation blighted plants were observed in row 1, while the row of control plants appeared healthy.

Eighty-nine days after inoculation a mottled leaf was removed from a blighted plant in row 1, and six plants in one pot in the greenhouse were inoculated with it by mashing the blighted tissues into the healthy leaves of the seedlings. Several pots of plants similar to the above were pricked with a flamed needle to serve as controls. Thirty-one days after inoculation the six plants were in the advanced stages of blight, but the control plants remained healthy. These results indicate that the disease is due to a specific virus which is capable of producing the characteristic symptoms of blight both in the greenhouse and the field plants.

One typical blighted plant from a large area was selected for inoculation purposes. This plant was mashed, and the juice strained through a cloth and used to inoculate one large potted plant in the greenhouse by needle pricks. A similar plant in another pot was pricked with a flamed needle and served as a control. Thirty days later the inoculated plant had developed typical symptoms of blight, while the control plant remained healthy. Nine days later individuals of *R. persicae*, which were known to be free from infection were placed on the diseased plant. Twenty-five days later aphids were removed from the above plant and placed on leaves of three large spinach plants growing in a field cage. Similar plants in the same field cage were used as controls. Twenty-one days later the control plants appeared healthy, but the three inoculated plants had developed typical symptoms of blight. These results indicate that the plants in the large area had typical spinach-blight, and that the virus from these plants produced the disease in greenhouse plants; further, that when aphids free from infection were allowed to feed on blighted greenhouse plants they were able to transfer the infectious entity to plants in field cages.

INOCULATIONS WITH A WATER SUSPENSION OF VIRUS FROM BLIGHTED PLANTS

A water suspension of blighted plants was made by mashing the mottled leaves in tap water. This suspension was strained through a cloth into an atomizer. With a flamed needle a row of plants in a field cage were pricked, and then sprayed with the contents of the atomizer. The wet leaves were again pricked with a flamed needle to insure the virus entering the tissues. Another row of plants in the same cage was pricked with a flamed needle and served as a control. Twenty days

after inoculation the first blighted plants were observed. All of the control plants appeared healthy. Twenty-seven days after observing the first blighted plants a mottled leaf was removed from one of them, and with it seedling spinach plants in a pot in the greenhouse were inoculated by mashing the diseased tissues into their leaves. A similar pot of seedlings was pricked with a flamed needle and served as a control. Thirty-one days later three of the seedling plants had developed decidedly mottled leaves, typical of blight. All of the control plants appeared healthy. These results indicate that the original blighted plants contained a virus which, when extracted in water and inoculated into other field plants in the outdoor cages, produced a condition similar to the original blighted plants. Leaves from these artificially infected plants contained a virus which produced typical symptoms of blight in plants growing in the greenhouse, thus indicating that it is not unfavorable soil nor temperature conditions which cause the disease in the field or in the greenhouse.

DIRECT INOCULATIONS WITH THE JUICE OF BLIGHTED PLANTS IN WHICH THE DISEASE HAD BEEN PRODUCED BY APHIDS

Blighted plants were collected from two large areas. Alate and apterous *Macrosiphum solanifolii* and *Rhopalosiphum persicae* were removed and placed on spinach seedlings in a field cage. Sixty-one days after transferring the aphids typical symptoms of blight appeared on two of the plants. Thirteen days later one more plant developed mottled leaves typical of blight. On the same day one mottled leaf was removed from each of the two blighted plants first observed in this cage, and was brought to the greenhouse for inoculation purposes. Ten spinach plants 49 days old were inoculated as follows: With a flamed needle, the juice from the mottled leaf of plant 1 was pricked into the healthy leaves of five seedlings in two pots, the other five seedlings in two pots being inoculated by mashing the blighted tissues into the leaves. Ten other spinach plants in four pots were inoculated in the same manner with a flamed needle and the mottled leaf from blighted plant 2. Two similar spinach plants in separate pots were pricked with a flamed needle to serve as controls.

Twelve days after inoculation, one of the potted plants had developed the mottled leaves characteristic of blight; the other inoculated plants and the controls appeared healthy. Sixteen days after inoculation a photograph was taken to show the difference in appearance between the inoculated plants and a control (Pl. 7, B). Twenty-four days after inoculation 13 of the 20 inoculated plants had developed mottled leaves characteristic of blight. The remaining inoculated plants, although not showing the mottled leaves, did not have the healthy appearance of the control plants.

These results indicate that the aphids which had fed on blighted plants carried the virus to healthy field plants. That the aphids were only carriers of the virus and not the true cause of the disease is shown by the fact that the virus from the plants artificially infected by the aphids produced typical symptoms of the blight when transferred by needle pricks to healthy plants in the greenhouse.

DIRECT VIRUS INOCULATIONS FROM FIELD TO GREENHOUSE AND BACK TO FIELD

Aphids were removed from blighted plants and placed on three pots of spinach seedlings. Subsequently the blighted plants were mashed in a mortar and the juice pressed out through a cloth. Some of this juice was pricked into seedling plants which were then placed under a globe in the greenhouse to keep them under more humid conditions. Twenty-one days after inoculation two of the plants had developed characteristic symptoms of the blight. A similar pot of plants, pricked with a flamed needle, covered with another globe, and used as a control, remained healthy. With some of the extracted juice, three plants in separate pots were inoculated by pricking the virus into the leaves. A similar pot of plants was pricked with a flamed needle to serve as a control. Eleven days after inoculation symptoms of spinach blight began to develop in three plants. Twenty-one days after inoculation, two of the plants were in advanced stages of blight, while the third was considerably stunted, but not as mottled as the two others. Eighty-six days after inoculation four large plants in a field cage were inoculated by mashing into them a leaf from one of the above blighted plants. Similar plants in the same cage were pricked with a flamed needle and served as controls. Eighteen days after inoculation three of the four plants had developed typical symptoms of blight, while the control plants appeared healthy.

DIRECT INOCULATIONS BY APHIDS PROVED BY VIRUS INOCULATIONS

Individuals of *Rhopalosiphum persicae* and *Macrosiphum solanifolii* were shaken from blighted plants and placed in a large pot containing over 100 spinach seedlings. Twelve days later distinct mottling of some of the spinach leaves was observed. Sixteen days after inoculation numerous plants had decidedly mottled leaves. Ten days later a large percentage of the 100 plants in this pot had developed typical symptoms of blight.

Twenty-three days after inoculation one leaf was removed from each of three blighted plants in the above pot, and with these mottled leaves seedlings in two pots were inoculated by pricking and mashing the blighted tissues into them. A third pot of six plants was inoculated by pricking juice from one of the blighted leaves into the seedlings. A

similar pot of seedlings was pricked with a flamed needle and served as a control. Seven days after inoculation the two pots, of two and four plants, respectively, developed typical symptoms of blight. Nine days after inoculation the six plants in one pot developed doubtful symptoms and five days later all of these plants had developed positive symptoms of blight. Twenty-one days after inoculation all of the above plants were in advanced stages of blight, while the control plants remained healthy. These results indicate that the aphids from the original blighted field plants served as carriers of the virus, because the plants which blighted as a result of the feeding of the aphids on them contained a virus which produced the disease when pricked into healthy spinach plants.

INOCULATIONS WITH THE TISSUES OF BLIGHTED PLANTS IN WHICH THE DISEASE WAS PRODUCED BY APHIDS

A number of *Rhopalosiphum persicae* and *Macrosiphum solanifolii* were removed from blighted plants and placed on a pot of seedling spinach 13 days old. This pot contained more than 100 seedlings. A similar pot of seedlings served as a control. Eighteen days after inoculation a few plants in the above pot had developed typical symptoms of blight. Thirty-four days after inoculation the majority of the inoculated plants had developed symptoms of the disease, while the control plants still appeared healthy. The majority of the inoculated plants eventually became much stunted and many died, but the control plants remained healthy (Pl. 8, A).

Eighteen days after inoculation two of the blighted plants were removed from the above pot and two seedling spinach plants were inoculated with them by pricking through the mottled leaves into the leaves of the seedlings with a flamed needle. A third plant in another pot was pricked with a flamed needle to serve as a control. These pots of plants were placed in a cage in the greenhouse. With the two blighted plants used for the greenhouse inoculations six plants in a field cage were inoculated by pricking through the blighted leaves into the leaves of the healthy plants. Seventy-nine days after inoculation four of the six caged field plants showed positive symptoms of spinach blight. Fourteen days after inoculation the two seedlings in the pot in the greenhouse had developed mottled leaves, while the control remained healthy. Eighty-four days after inoculation a photograph was taken to show the appearance of the control as compared with the inoculated plants (Pl. 8, B). These results indicate that spinach-blight is due to a specific virus which is readily transmitted from the field to the greenhouse, and vice versa, either by means of aphids which have fed on diseased plants or by transferring the juice of the diseased plants by needle pricks.

EARLY FIELD EXPERIMENTS[1] ON THE INSECT TRANSMISSION OF SPINACH-BLIGHT, 1916–17

The following data were collected from experiments performed in December, 1916, and January, 1917. The plants used in the experiments had been growing in large field cages for about three weeks previous to the time the inoculations were made. Large-sized lantern globes (Pl. 10, A) were used to cover the plants during the 48-hour period which the aphids were allowed to remain on them. Adult apterous females were used in the transfers of aphids from the diseased to healthy plants.

DIRECT TRANSFERS OF APHIDS FROM DISEASED TO HEALTHY PLANTS

Series 1

Inoculations with Macrosiphum solanifolii.—Three plants in a field cage were selected and on the first were placed 10, on the second 10, and on the third 20 individuals of *M. solanifolii* which had previously been feeding on diseased spinach. Positive symptoms of blight developed on all three plants in 22, 17, and 22 days, respectively. The mean temperature inside the cages for the 22 days of the incubation period was 45° F. Outside the cages the mean temperature was 42°.

Inoculations with Rhopalosiphum persicae.—Another series of three healthy plants were inoculated by placing on them 10, 20, and 10 individuals of *R. persicae*, respectively, from diseased plants. The typical mottling and malformations due to the disease appeared on all three of the plants 22, 29, and 22 days after the transfers were made. This experiment was conducted in the same cage as the first. No disease appeared on four untreated plants used as controls.

Series 2

Series 2 was started in January, 1917, and the results are shown in Table I. On January 17 thirty adults of *Macrosiphum solanifolii* which had been feeding on lettuce were placed on plant 1 of series 1, and 30 adults of *Rhopalosiphum persicae* were placed on plant 4 of the same series. The aphids were allowed to feed for three days on these plants before the transfers were made. Individuals of *M. solanifolii* from diseased plant 1 were placed on three healthy plants. After feeding for 48 hours the aphids were removed. Positive symptoms of the disease developed in three of the plants 16, 16, and 23 days after inoculation. Three plants were inoculated by needle pricks with virus taken from plant 1. All three plants gave positive symptoms of the disease on the sixteenth day. Ten adults of *M. solanifolii* born and reared on lettuce (*Lactuca sativa*) were placed on each of three healthy spinach

[1] During the summer of 1916 cooperative experiments were planned between the Entomologist of the Virginia Truck Experiment Station and Mr. D. E. Fink, of the Office of Truck Crop Insect Investigations of the Bureau of Entomology. This work was started in the fall of 1916 on a spinach field on the Station farm. Successful transmissions of blight from diseased to healthy plants were obtained, and some data relative to the character of spinach blight collected.

seedlings. After remaining on the plants for 48 hours, the aphids were removed. No cases of blight had developed at the time the experiment closed, 54 days after the transfers were made, thus indicating that the aphids from lettuce did not carry infection. Ten control plants which were untreated remained healthy. Ten adults of *R. persicae* taken from plant 4 were placed on three healthy spinach plants. All three developed blight on the sixteenth day. Three plants were inoculated by needle pricks with the virus from plant 4. Two of the plants developed positive symptoms of the disease on the fifteenth day. The third remained healthy until the experiment closed. Forty adult *R. persicae*, the offspring of individuals which had been feeding on lettuce for three generations, were distributed equally on four healthy spinach plants. They remained on these plants for a period of 48 hours and were then removed. One plant developed typical symptoms of blight 23 days after it was inoculated. The three remaining plants were still healthy when the experiment closed. Ten plants which were untreated and used as controls remained healthy.

TABLE I.—*Results of field experiments on the insect transmission of spinach-blight (series 2), Norfolk, Va., 1917*

Plant No.	Source of diseased material.	Method of transmission.	Species of insect used.	Incubation period.	Mean temperature in cages during incubation period.
				Days.	°*F*
1	Plant 1, first series 1916 experiments.	10 live aphids	*Macrosiphum solanifolii.*	16	46
2	do	8 live aphids	do	16	45
3	do	12 live aphids	do	23	43.2
4	do	Needle pricks with diseased tissue.		16	45
5	do	do		16	45
6	do	do		16	45
7	Controls untreated			Healthy.	
8	do			do	
9	do			do	
10	Aphids reared for two generations on lettuce.	10 live aphids	*M. solanifolii.*	do	
11	do	do	do	Healthy.	
12	do	do	do	do	
14	Plant 4, first series	do	*Rhopalosiphum persicae.*	16	45
15	do	do	do	16	45
16	do	5 live aphids	do	16	45
17	do	Needle pricks		15	45
18	do	do		15	45
19	do	do		Healthy.	
20	Controls untreated			do	
21	do			do	
22	do			do	
23	Aphids, third generation, on lettuce.	10 live aphids	*R. persicae.*	23	43.2
24	do	do	do	Healthy.	
25	do	do	do	do	
26	do	do	do	do	

The results obtained in these early experiments indicated that the pathogenic agent of spinach-blight is extremely virulent and can easily be transmitted from one plant to another by aphids which pass from a diseased to a healthy plant. It is also transmissible to healthy plants by means of needle-prick inoculations. The incubation period, or the time elapsing from the inoculation to the appearance of color changes in the host, varied from 16 to 23 days, under the conditions present. One case of blight appeared on a plant to which lettuce-fed aphids had been transferred. Similar infections occurred about the same time in another set of field experiments being conducted in other cages.

The following field experiments were begun in 1916. Holland-grown seed was sown in December in field cages. The seedlings had developed the second pair of true leaves at the time the inoculations were made. The diseased material used for inoculation purposes was collected from a typical diseased area, located on a farm about ½ mile distant from the Station property. Adult apterous individuals of *Macrosiphum solanifolii* and *Rhopalosiphum persicae* were transferred to the healthy plants. The inoculation period or the length of time the aphids remained on the healthy plants was 48 hours in each case. The methods employed in transferring the aphids and in making the inoculations are discussed elsewhere. The inoculations in the following experiments were made on January 10, 1916, and the results are given in Table II.

TABLE II.—*Results of field experiments on the insect transmission of spinach-blight at Norfolk, Va., 1916–17*

Method of inoculation.	Species used.	Number of insects per plant.	Number of plants used.	Number of plants infected.	Percentage of infection.	Average period of incubation.
						Days.
Live virus-bearing aphids	*Macrosiphum solanifolii.*	10	12	11	91.6	17.8
Do	*Rhopalosiphum persicae.*	10	12	11	91.6	17
Juice of virus-bearing aphids pricked into healthy plants.	*M. solanifolii*	......	12	6	50	21.1
Do	*R. persicae*	......	12	3	25	24.6
Virus pricked into healthy plants.	....	......	12	9	75	20.6
Pricked with sterile needle	Controls	......	12	0	0	0
Nonvirus-bearing aphids born and reared on lettuce.	*M. solanifolii*	10	10	2	20	25.5
Nonvirus-bearing aphids born and reared on spinach.	*R. persicae*	10	10	3	30	27
Control plants	Uninoculated	......	84	0	0	0

DIRECT TRANSFERS OF MACROSIPHUM SOLANIFOLII FROM DISEASED TO HEALTHY PLANTS

On each of 12 vigorous plants growing in field cages were placed 10 adults of *Macrosiphum solanifolii* from diseased plants. Eleven of the twelve plants developed positive symptoms of the disease. The incubation period varied from 12 to 30 days, the average being 17.8 days. One plant remained healthy until March 2, when the last record was made.

DIRECT TRANSFERS OF RHOPALOSIPHUM PERSICAE FROM DISEASED TO HEALTHY PLANTS

Another series consisted of 12 plants inoculated in a similar manner, except that 10 adults of *Rhopalosiphum persicae* were placed on each of 12 healthy plants. The aphids were collected on the same diseased plants from which a number of *Macrosiphum solanifolii* were taken for use in the first series. Again eleven plants developed symptoms of the disease between 12 and 30 days after they had been inoculated. The average incubation period was 17 days, or 0.8 day less than for the *M. solanifolii* series.

INOCULATIONS WITH THE JUICE OF CRUSHED APHIDS COLLECTED FROM DISEASED SPINACH

Twelve healthy plants were inoculated with the juice of crushed *Macrosiphum solanifolii* collected on diseased plants. The inoculations were made by means of needle pricks. Six plants became infected in an average period of 21.1 days. Likewise 12 plants were inoculated with the juice of crushed *Rhopalosiphum persicae* from diseased plants. These innoculations resulted in three infections in an average time of 24.6 days.

INOCULATIONS WITH VIRUS FROM PLANTS UPON WHICH THE APHIDS FED

Another series f 12 plants were inoculated with the virus taken from the crushed leaves of diseased plants upon which the aphids were feeding before they were transferred to healthy plants. The virus was pricked into the leaves by means of a sterile needle. Nine plants developed symptoms of blight in an average time of 20.6 days. As a check on this series 12 plants were pricked with a sterile needle. No cases of infection resulted, and the plants remained healthy until the experiment was closed.

TRANSFERS OF APHIDS BORN AND REARED ON LETTUCE TO HEALTHY SPINACH

As a control on the previous transfers made with insects from diseased spinach, 10 healthy plants were selected, and on them were placed a number of adults of *Macrosiphum solanifolii* born and reared on lettuce, and which had not fed on spinach until transferred to the plants in this series. The parents of these aphids were collected in the field from supposedly healthy spinach. Two of the 10 plants developed positive symptoms of blight, the first appearing on the twenty-first and the second on the thirtieth day after the transfers had been made. The eight

remaining plants were healthy on March 2. A duplication of this experiment was made with *Rhopalosiphum persicae* which had been born and reared on supposedly healthy spinach in the greenhouse. The parents of these aphids were collected from a field of apparently healthy spinach which at the time gave no evidence of the presence of the blight. The aphids were placed on spinach in the greenhouse and allowed to remain on the seedlings until there was a sufficient number of adults to use in this experiment. Four days after the transfers had been made to the outdoor cages, or 29 days after the original aphids had been brought to the greenhouse, six of the seedlings on which they had been placed became diseased. In the outdoor series three plants were infected, the symptoms developing in an average time of 27 days.

About 10 plants in each of the field cages remained untreated and were used as controls. There were 84 plants used in this manner, and all remained healthy until the experiment closed on March 2.

The results obtained through the transfers of live aphids from diseased to healthy plants proved the ability of the aphids to transmit the disease from plant to plant in the field. The ability of *Macrosiphum solanifolii* and *Rhopalosiphum persicae* to transmit the disease was about the same, although the incubation period for plants inoculated by *M. solanifolii* was 0.8 day longer than the average incubation period of plants inoculated by *R. persicae*. The juice obtained from crushed aphids from blighted plants proved to be infectious; that obtained from *M. solanifolii* gave 50 per cent and that from *R. persicae* 25 per cent of positive infections. It is noticeable that in this case the average incubation period was one to seven days longer than where the inoculations were made through either the feeding punctures of the aphids or by pricking the expressed virus into the plant tissues. The peculiar results which were obtained by the transfers of lettuce-fed aphids to healthy plants are not easily explained. It was known that the *M. solanifolii* had not come in contact with diseased spinach from the time of their birth until they were transferred to the healthy spinach plants, and yet they produced positive cases of blight in 2 cases out of 10 inoculations. *R. persicae* from supposedly healthy spinach plants produced 3 cases of blight out of the 10 plants inoculated. It will be noticed that the average incubation period of the disease in all five of these positive cases was 8 to 10 days longer than the average incubation period of the disease when caused by the direct transfers of aphids from diseased plants. During the experiment it was supposed that in the series of *R. persicae* there may have been a latent case of blight in the healthy spinach from which the aphids had previously been taken in the field and that the virus had been carried from it to the experimental plants. The possibilities of seed or soil infection were eliminated, as thousands of plants were growing at the time under insect-free conditions though in soil known to have grown diseased spinach. No cases of blight developed on any of these plants.

The control plants in all of the cages were free from blight; therefore it was thought either that lettuce was an alternate host of the inciting factor of the disease or that in some unknown manner the plants became infected during the manipulation incidental to the transference of the aphids.

In order to check these points, a duplication was made of the transfers of the *Macrosiphum solanifolii* from lettuce to spinach. In this series 20 spinach plants were used, and 10 adult aphids born and reared on lettuce were placed on each plant, as in the original series. Every precaution was taken to prevent the infection of the plants in any other manner than through the agency of the aphids. Twenty additional plants used as controls were untreated. Twenty plants were inoculated with the juice obtained by crushing the lettuce leaves from which the aphids had been taken. Four cases of blight developed in 22, 22, 29, and 36 days, respectively, after inoculation among the plants upon which the aphids had been allowed to feed. All the untreated plants remained healthy, as did those which were prick-inoculated with the juice of lettuce leaves. Twenty control plants pricked with a flamed needle remained healthy.

Twenty healthy spinach plants were inoculated with the juice of crushed *Macrosiphum solanifolii* taken from the original lettuce plants. Ten plants were pricked with a sterile needle and used as controls. Two of the inoculated plants developed positive symptoms of the disease in 22 and 26 days, respectively. The 18 other plants in the series, together with the control plants, remained healthy until the experiment closed, 54 days later. Another series of inoculations was made with the juices of aphids which had been feeding on lettuce, eggplant (*Solanum melongena*), and peppers (*Capsicum* spp.), to inoculate healthy spinach plants.

In all cases *M. solanifolii* was used. The parent aphids were collected in the field from lettuce plants, brought to the greenhouse, and placed on the various food plants, on which they were allowed to remain until the first-generation offspring had been produced. The inoculations were made about the time the majority of the first generation had reached the fourth instar. The following results were obtained.

Forty spinach plants inoculated with the juice of aphids collected from lettuce gave one positive infection, and the plant developed typical blight. Twenty plants were inoculated with the juice of aphids reared on eggplants; two positive infections resulted. Twenty plants inoculated with the juice of aphids reared on peppers remained healthy. Forty plants were pricked with a sterile needle and remained healthy. Three lots of 10 plants each were inoculated with the juice of crushed lettuce leaves, eggplant leaves, and pepper leaves. These plants remained healthy.

The results obtained in these series indicate the improbability of lettuce or eggplant serving as alternate hosts of the virus. The possibility of experimental error or outside infection was also rendered unlikely through the various duplications of the work. The fact remains that aphids which had never come in contact with spinach produced a small percentage of infections when placed on healthy spinach plants; also, the juice of aphids similarly treated produced the disease in a few cases when inoculated into healthy plants.

NATURAL INFECTION OF SPINACH PLANTS

NATURAL INFECTION OF GREENHOUSE SEEDLINGS BY VIRUS-BEARING APHIDS

Blighted spinach plants were collected, transplanted to a bench in the greenhouse, and covered with a large glass cage. Some American-grown spinach seed which had been soaked for a few minutes in 1 to 100 formaldehyde was planted in this cage. Ten days after transplanting the blighted plants to the greenhouse cage it was observed that they were dying and that the aphids from these plants had crawled to the seedling plants which had come up about six days after planting the seed. Only the cotyledon leaves had developed on the seedling plants at that time. The aphids in this cage were killed by fumigation a short time thereafter and the transplanted blighted plants died. About 30 days after the seedlings had come up it was observed that some of them had developed characteristic symptoms of blight, and within the next 10 days the majority of the plants had developed striking symptoms. This included some 25 plants in all. About 40 days after the seedlings had come up, the aphids in this case having been killed by fumigation, a pot of 12 seedling plants was set in this cage among the blighted plants. Thirty-five days later none of the seedlings in this pot had developed any symptoms of blight, although they were surrounded by blighted plants. This indicates that blight is not transferred except by aphids or some mechanical means. After making this observation, individuals of *Rhopalosiphum persicae* known to be free from infection were transferred to this cage. Twenty-nine days later the majority of the plants in the pot of healthy seedlings previously transferred to this cage had developed characteristic symptoms of blight, thus indicating that the aphids had traveled and carried infection from the blighted to the healthy plants in the pot.

A number of *R. persicae* from blighted plants in the above cage were transferred to large plants in a field cage. Similar plants in the large cage were used as controls. Thirty-one days after they had been inoculated all of the plants had developed characteristic symptoms of blight, while the control plants remained healthy. These results substantiate those of previous experiments to the effect that spinach-blight is a specific disease which may be produced either in the field or in the greenhouse,

and may be readily transferred from the field to the greenhouse, and vice versa, by means of the virus causing this disease.

Blighted plants from the Station field were transplanted to pots of greenhouse soil and placed in cages. About these were placed pots of seedling spinach. Care was used that the leaves of the seedlings did not come in contact with the blighted plants. The next day it was observed that aphids had crawled from the blighted plants to the seedlings. Thirteen days later definite symptoms of blight were observed on these plants. These data indicate that aphids on the blighted field plants served as carriers of the blight to the seedlings and that the spinach-blight in the Station field was the same as that which had appeared in the fields on other farms.

To prove that the aphids serve as carriers of the virus and not as the cause of the blight, mottled leaves from the smaller of the above transplanted, blighted, field plants were used to inoculate four pots of spinach seedlings by mashing the blighted tissues into the leaves of the seedlings. Two similar pots of seedlings were pricked with a flamed needle to serve as controls. Eight days after inoculation seedling plants in all four of the above pots had developed mottled leaves characteristic of blight, especially about the points of inoculation. Thirteen days after inoculation the majority of these plants had developed typical symptoms of blight. Twenty-six days after inoculation practically all of the inoculated plants were in advanced stages of spinach-blight, while the controls remained healthy.

Blighted plants were collected from the Station field and brought to the laboratory, where aphids were allowed to crawl from them to a pot of 50 or more healthy seedlings. The next day it was observed that numerous aphids were present on the seedlings. Therefore the blighted plants were removed from the cage. Twenty-one days after inoculation some of the plants in this pot of seedlings had developed the mottled leaves characteristic of blight. Five days later a majority of the 50 or more plants developed typical symptoms of blight. The aphids originally transferred to this pot were killed by fumigation 31 days after inoculation. *Rhopalosiphum persicae* known to be free from infection were transferred to these plants. Numerous seedling plants were coming up in this pot from seed planted after the original aphids had been killed. The majority of these secondary seedlings eventually blighted, thus indicating that the aphids had become virus bearers and had transferred the inciting entity to the seedlings.

Ten days after transferring the *Rhopalosiphum persicae* to the above pot of seedlings, a blighted leaf bearing both larval and adult aphids was removed from one of the plants and placed on a large plant in a field cage. Numerous spinach seedlings from seed planted somewhat later were growing about this plant. Thirty-two days after inoculation the large plants and two of the seedlings had developed positive symptoms

of blight. The plants left as controls appeared healthy. These results indicate that spinach-blight is caused by a definite virus, and is transmitted from plant to plant by the aphids.

COMPARISON OF DISEASED MATERIAL FROM VARIOUS LOCAL SOURCES

FARM C.—Diseased plants collected in two blighted areas on farm C were placed in a field cage, care being taken that the blighted plants did not touch the healthy spinach seedlings in the cage. Both species of aphids were present on the blighted plants. Sixty-one days later two of the seedling plants had developed the mottled leaves typical of spinach-blight. Twelve days later a third seedling plant developed definite symptoms of blight.

These results indicate that the aphids on introduced, blighted plants had crawled to the seedling plants and had infected them with the spinach-blight virus.

In order to prove the infection, one mottled leaf was removed from each of two diseased plants for prick inoculations. With a flamed needle the leaves of two spinach plants were pricked to serve as controls. Seven plants in two pots were inoculated by pricking through the mottled leaf of plant 1 into the healthy leaf of the seedling. Seven similar plants in two pots were inoculated by pricking into them the juice of the mottled leaf from plant 2. Twelve days after inoculation five plants developed typical symptoms of blight. Thirty-six days after inoculation 12 of the 14 inoculated plants had developed the mottled leaves characteristic of blight, but the control plants were large and apparently healthy. The other inoculated plants, although not showing typical symptoms of blight, did not have the healthy appearance of the control plants. These results indicate that the aphids are carriers of the virus, and that spinach-blight is caused by a specific virus which can be transferred from plant to plant by needle pricks as well as by aphids.

STATION FARM.—Blighted plants were collected from the Station field and piled on the ground in the edge of the woods near the greenhouse. Pots of spinach seedlings from seed from six different sources were placed in wire cages so as to have seed lots 1, 2, and 3 in one cage and 4, 5, and 6 in another. Two such series were made, a total of 12 pots in four cages. Some of the blighted plants from the above-mentioned pile were placed in each cage, care being exercised that the blighted plants did not touch the seedlings in the pots. The next day it was observed that *Macrosiphum solanifolii* and *Rhopalosiphum persicae* had crawled from the leaves of the blighted plants onto the leaves of the seedling plants; therefore the blighted plants were carefully removed from the cages so that they did not touch the seedlings. Six days later seedlings in seed series 1 and 5 had the mottled leaves characteristic of blight, and 11 days later a large percentage of the seedlings in each of the seed

series showed mottled leaves; but the controls remained healthy. These pots were kept under observation for the next month, and during this time the majority of the seedlings in all of the seed lots became mottled and stunted in growth, thus indicating that it made little difference as to the source of seed of the Savoy strain of spinach grown from commercial seed as regards the resistance to blight.

Farm D.—Blighted plants were collected from two fields on farm D and aphids from some of these plants were placed on a large pot containing about 100 spinach plants 13 days old. A similar pot of seedlings served as a control. Eighteen days after inoculation some of these seedling plants had developed the mottled leaves typical of blight. Twenty-nine days later some of the blighted plants were alive, but in advanced stages of the disease. Control plants remained healthy. These results indicate that diseased plants scattered over fields are caused by the same virus which produces large areas of blighted plants.

Farm B.—Blighted plants were collected from farm B and brought to the Station, where a number of *Rhopalosiphum persicae* and *Macrosiphum solanifolii* were removed from them and placed on healthy spinach plants growing in a field cage. The blighted plants were then placed in another field cage, care being taken not to allow them to come in contact with the seedlings nor to scatter any of the remaining aphids on the seedlings. A similar cage of field plants served as a control. Sixty-three days after inoculation, blighted plants were observed in both of the above cages, while the control plants remained healthy. These results indicate that the aphids served as carriers of the blight virus, it making little difference whether they were placed directly on the healthy plants or allowed to travel by themselves from the blighted plants to the healthy seedlings. Mottled leaves were removed from blighted plants 22 days after the disease had been first observed in the cages, and seven plants growing in two pots in the greenhouse were inoculated by mashing the diseased tissues into the healthy leaves of the seedlings. A similar pot of plants pricked with a flamed needle served as a control. Eight days after inoculation two of the plants had developed the mottled leaves characteristic of blight, while the five others appeared doubtful. Thirty-eight days after inoculation all seven of the plants had developed typical symptoms of blight, but the control plants remained healthy.

Similar results were obtained by inoculating potted plants in the greenhouse with a leaf from a blighted plant from the other field cage. These results indicate that the aphids carried the blight virus from the original field plants to the caged plants and that this virus was readily carried from a blighted, caged plant to the greenhouse plants by needle pricks.

Farm E.—On October 21, 1916, spinach seed was planted on a field where spinach had not been grown for some time. A characteristic large blighted area subsequently developed at one side of this field. On Janu-

ary 26, 1917, this area was photographed (Pl. 9, A). According to information obtained from the grower, spinach-blight began to develop in this area during the first week in January. Besides this large area, a few smaller areas also developed in the same field. Blighted plants were collected from the largest area and brought to the greenhouse, where individuals of *Macrosiphum solanifolii* and *Rhopalosiphum persicae* were removed and placed on six pots of spinach seedlings 1 month old. Similar plants were kept in another cage as controls. Nineteen days after inoculation the majority of the plants in each of the pots had developed characteristic symptoms of blight. Some of the plants from which the aphids had been removed were placed in a cage with six pots of spinach seedlings, care being taken that the blighted plants did not come in contact with the seedlings. The next day it was observed that many of the aphids had crawled to the seedling plants. Nineteen days after inoculation numerous blighted plants were observed among the pots of seedlings. The controls appeared healthy. These results indicate that the blighted plants on farm E were produced by the same specific virus which caused blight on other farms.

A few of the central leaves were removed from blighted field plants from which the aphids had been taken, and six pots of seedlings were inoculated by mashing the blighted tissue into the leaves. Seventeen days after inoculation, some of the plants in each of the above pots had mottled leaves. Forty days after inoculation plants in each of the pots were in the advanced stages of blight, while the control plants appeared healthy.

Forty-three days after inoculation a mottled leaf was removed from one of the blighted plants, and five healthy seedlings in another pot were inoculated by mashing the diseased tissues into them. Eighteen days later two of the plants had developed the mottled leaves of blight. The control plants appeared healthy.

With another mottled leaf, removed from a blighted plant 43 days after inoculation, five large plants in a field cage were inoculated by mashing the diseased tissues into the healthy plants. Similar plants in the same cage were pricked with a flamed needle and served as controls. Seventeen days after inoculation all five of the above plants had developed decidedly mottled leaves, but the control plants appeared healthy. These results show that the spinach-blight on this farm is due to a specific virus which may be transferred from diseased to healthy plants either by aphids or by needle pricks, thus indicating that it is the same disease as that occurring on other farms.

FARM F.—In a field of spring spinach on farm F located at least 10 miles from the Experiment Station a few scattered plants appeared to be affected with blight. Some of these were collected and brought to the greenhouse, where aphids were removed and placed on two pots of spinach seedlings. Similar pots of plants were used as controls. *Macro-*

siphum solanifolii were more abundant than *Rhopalosiphum persicae*. Two other pots of seedlings were inoculated by mashing diseased tissues from the blighted plants into the leaves of the seedlings. Nineteen days after inoculation one of the inoculated plants had developed characteristic symptoms of the blight. One mottled leaf was removed from this plant, and with it three seedlings in another pot were inoculated by mashing the diseased tissues into their leaves. With another leaf from the blighted plant inoculations were made in one of the field cages by mashing the blighted tissues into the leaves of the plants. Twenty-five days after the inoculation typical symptoms of blight appeared on eight plants in the field cage, while the control plants remained healthy. Farm F is located a considerable distance from the majority of the farms where the blighted plants used in the other experiments had been obtained. The fact that virus from the diseased plants on this farm produced blight in both greenhouse and field-grown plants indicates that the disease is the same as that occurring on the other farms.

COMPARISON OF SCATTERED, INDIVIDUAL BLIGHTED PLANTS WITH BLIGHTED PLANTS FROM AREAS

Further experiments were begun with the object of determining any variation in the nature and transmissibility of spinach-blight occurring in individually diseased plants and plants in diseased areas. Both species of aphids were used to make the transfers, and the material was checked each time by needle-prick inoculations. When transfers were made, an average of four insects were placed on each plant Twenty-four hours later the plants were fumigated to kill the aphids. The healthy plants were 24 days old at the time of inoculation. The diseased material used for inoculation purposes was collected on various farms in the spinach-growing region of eastern Virginia. The results of this experiment are given in Table III. These show that the percentages of infection are similar for the individually diseased plants and for those collected in the diseased areas. This indicates that the factors producing the disease from both of the above sources are the same.

Since spinach-blight occurs both in scattered plants in the field and in distinct areas, it led to a study of the cause of these variations. Briefly stated, the results of this study indicated that under certain conditions favorable to the development of aphids there often occur heavy local infestations. During such periods the apterous females are particularly numerous and active, passing readily from plant to plant. Provided the favorable conditions persist, the infested area may be enlarged from a few plants to an area many yards in diameter. If a blighted plant occurs near the center of infestation, infection may be carried by the aphids to numerous other plants. When the conditions change and the aphids are greatly reduced in numbers, the spread of infection is likewise checked. Thus is an area of blighted plants formed. The infec-

tions in the individually diseased plants which occur scattered through out the fields are probably produced by the alate female aphids which fly from blighted plants carrying the infection to healthy plants upon which they alight and feed. These scattered, blighted plants in turn may serve as centers of infection for future diseased areas.

TABLE III.—*Transmission of spinach-blight from various local sources, Norfolk, Va., 1917*

Species of aphid used.	Source of diseased material.	Number of plants inoculated.	Number of plants infected.	Average length of incubation period.	Number of insects per plant.
				Days.	
Macrosiphum solanifolii...	Farm A (center of diseased area).	10	9	16	4
Do.................	Farm B (center of diseased area).	10	10	17	4
Do.................	Farm C (center of diseased area).	10	8	14.3	4
Do.................	Station farm (center of diseased area).	10	9	16.2	4
Rhopalosiphum persicae...	Farm A (center of diseased area).	10	6	17	4
Do.................	Farm B (center of diseased area).	10	8	17.7	4
Do.................	Station farm (center of diseased area).	10	9	16	4
Macrosiphum solanifolii...	Farm A (individually diseased plant).	10	8	16.4	4
Do.................	Farm B (individually diseased plant).	10	8	14	4
Do.................	Station farm (individually diseased plant).	10	9	17.3	4
Rhopalosiphum persicae...	Farm A (individually diseased plant).	10	6	19.2	4
Do.................	Farm B (individually diseased plant).	10	7	16.1	4
Controls.................	Untreated, 20, all healthy....	.....	.....	.....	.....

EXPERIMENTS ON THE INSECT TRANSMISSION OF SPINACH-BLIGHT, 1916–17

RELATIVE INFECTIVITY OF APHIDS OBTAINED FROM PLANTS IN VARIOUS STAGES OF THE DISEASE

A series of experiments was begun on January 12, 1917, to determine the relative infectivity of the virus from plants in various stages of spinach-blight. As has been discussed, there are eight arbitrary but rather definite stages of advancement of the disease between the first appearance of pathogenic symptoms and the ultimate death of the plant. In order to make this test, it was necessary to collect typically diseased field plants in the various stages. A large number of lettuce-fed aphids were placed on the diseased plants and allowed to feed for three days before transferring them to healthy spinach plants. Both species of

aphids were used in the transfers, and inoculations were also made with the virus obtained from the blighted plants. The first to sixth stages, inclusive, were used. Plants in the seventh and eighth stages are more or less dried, withered, and rarely serve as food for aphids in the field. Ten aphids from diseased plants were transferred in each case and remained on the healthy plants for 48 hours.

TABLE IV.—*Relative infectivity of aphids obtained from plants in various stages of spinach-blight at Norfolk, Va., 1917*

Source of material used in making inoculations.	Number of plants inoculated by the transference of insects.	Number of infections.	Average length of period of incubation.	Number of plants inoculated with virus.	Number of infections produced.	Average length of period of incubation.
			Days.			*Days.*
Plant in sixth stage of disease	4	4	13	2	2	15
Plant in fifth stage of disease	4	4	14.5	2	1	17
Plant in fourth stage of disease	4	4	14	2	2	19
Plant in third stage of disease	4	4	19	2	1	21
Plant in second stage of disease	4	4	22	2	2	22.5
Plant in first stage of disease	4	2	25.5	2	1	30
Healthy plant	4	0	0	2	0	0

RELATION BETWEEN LENGTH OF INOCULATION PERIOD AND NUMBER OF INFECTIONS PRODUCED

From Table IV it will be seen that the ratio between the inoculations and infections obtained were about the same for all the stages between the second and sixth, inclusive, which would indicate that the virus from the various stages is approximately equally infectious. The most striking results obtained were the variation in the incubation period of the disease in the inoculated plants. The incubation period in the plants inoculated by insects transferred from the sixth-stage-diseased plants averaged 13 days and in the plants inoculated directly with the virus by needle pricks it was 15 days. For plants inoculated by insects from the fifth-stage-diseased plant the incubation period of the disease was 14.5 days. Direct needle-prick inoculations with the virus from the same source resulted in an average incubation period of 17 days. The incubation period gradually increased in length with the fourth, third, and second stages until in the first stage it had reached a length of 25.5 days before the disease was produced by insects and of 30 days before the disease was produced by direct needle-prick inoculation with the virus. These figures indicate that, while the virus from the various stages may be about equally infectious, so far as the ability to produce the disease is concerned, yet owing perhaps to the concentration of the infective entity in the plant juices, which increases as the disease advances, the virus of the more

advanced stages of the blight produced positive symptoms in about half the time that was required to produce them, when plants were inoculated with the virus obtained from plants in the early stages of the disease. It is also worthy of note that under conditions present in this experiment, when 10 adult aphids were transferred from a diseased to a healthy plant, positive symptoms of the disease developed in two to five days' less time than when the inoculation was made with the same diseased material by means of the expressed virus pricked into the plants with a sterile needle. No infections were obtained in the transfers of lettuce-fed aphids to healthy spinach, and no infections were obtained when prick inoculations were made with the juice obtained from healthy spinach plants.

In order to determine the approximate length of time which aphids from blighted spinach must remain on healthy plants to produce infections, aphids from diseased plants were transferred to a series of healthy plants and allowed to remain on them for various periods of time. Since infections were previously obtained by allowing the insects to remain on plants for 48 hours, the length of the periods in the present series were shortened to 5 minutes, 2 hours, 14 hours, and 24 hours. As the aphids had been disturbed, they fed little during the 5-minute period that they were allowed to remain on the healthy plants. They were observed occasionally to plunge their beaks into the plant tissues for an instant, quickly withdrawing them, moving to some other part of the plant and repeating the performance. The results of this experiment are given in Table V.

TABLE V.—*Results of experiments on the relation between the length of the inoculation period and the number of infections produced*

Length of time aphids remained on the plants.	Species used or treatment.	Number of plants inoculated.	Number of plants infected.	Average length of incubation period.	Number of plants remaining healthy.
				Days.	
24 hours	*Macrosiphum solanifolii*	14	11	15.2	3
14 hours	do	19	15	18.8	4
2 hours	do	9	6	16.3	3
5 minutes	do	8	4	24	4
24 hours	*Rhopalosiphum persicae*	17	12	16.3	5
14 hours	do	13	8	15	5
2 hours	do	8	5	14	3
5 minutes	do	9	2	27.5	7
	Plants inoculated with virus from the original diseased plant	10	8	16.1	2
Controls	Untreated	20			20

Where the aphids remained on the plants for only 5 minutes less infections were obtained than when they fed on the plants for 2 hours or longer. The percentage of infections which resulted from the longer

periods of inoculation varied from 50 to 75 per cent, but apparently had no direct relationship with the length of time the aphids had been on the plant. Little difference could be observed in the infectivity of the two species. The length of the incubation period of the blight showed but slight variation in the various plants affected, 14 to 18.8 days, and there was no indication of a relationship between its length and the length of the inoculation period. The shortest average incubation period was obtained with individuals of *Rhopalosiphum persicae* placed on the plants for 2 hours, while the longest was when *Macrosiphum solanifolii* were placed on the plants for 14 hours. When a number of *M. solanifolii* were allowed to remain on the plants for 5 minutes 4 plants out of 8 became infected and showed positive symptoms on the twenty-fourth day. Where *R. persicae* were used, 2 plants out of 9 showed positive symptoms in an average time of 27.5 days. Of 10 plants inoculated with the virus of the original diseased plant from which the aphids were taken, 8 became infected and developed positive blight symptoms in an average time of 16.1 days. Ten untreated plants used as controls remained healthy; hence, it will be seen that virus-bearing aphids produce infections when they feed on healthy plants for only a few minutes.

INFECTIVITY OF MATURE AND IMMATURE APHIDS

The following series of transfers were made in order to determine the relative infectivity of aphids in their various stages of development. These were performed with both *Macrosiphum solanifolii* and *Rhopalosiphum persicae*. The insects were reared and allowed to feed, reproduce, and develop on typical fourth-stage-diseased spinach plants. Individuals representing each of the five instars were transferred to series of healthy plants. Transfers were also made with adult alate and apterous females. A series of 10 plants were inoculated with insects in each instar, four insects to each plant. They were allowed to remain on the plants for a period of 48 hours. As will be seen from Table VI, those individuals of *M. solanifolii* of the first instar which had been transferred directly from the diseased to the healthy plants produced 1 infection in 10 inoculations, positive symptoms appearing on the twenty-second day (Pl. 9, B). The remaining plants were healthy when the experiment closed, 54 days later. The second instar of *M. solanifolii* produced 4 infections out of 10 inoculations, positive symptoms appearing in an average time of 19 days. The third instar of *M. solanifolii* produced 4 infections in an average time of 18.5 days. The fourth instar of *M. solanifolii* produced 8 infections in an average time of 17.6 days. The fifth instar, alate form, produced 9 infections in 17.2 days, while the fifth instar, apterous form, produced 7 infections in an average time of 14 days. It will be seen that when healthy plants are inoculated by transferring to them adult aphids from diseased plants, the incubation period of the disease is several days shorter than it is where aphids in

the first or second instars are used. The percentage of infections also is increased according to the age of the aphids when they are transferred from disease to healthy plants.

TABLE VI.—*Infectivity of mature and immature aphids*

Species.	Instar.	Number of insects to plant.	Number of plants inoculated.	Number of plants infected.	Average length of incubation period.	Number of plants remaining healthy.
					Days.	
Macrosiphum solanifolii	First	4	10	1	22	9
Do	Second	4	10	4	19	6
Do	Third	4	10	4	18.5	6
Do	Fourth	4	10	8	17.6	2
Do	Fifth alate	4	10	9	17.2	1
Do	Fifth apterous	4	10	7	14	3
Rhopalosiphum persicae	First	4	10	......	......	10
Do	Second	4	10	3	18.2	7
Do	Third	4	10	4	19	6
Do	Fourth	4	10	6	16.3	4
Do	Fifth alate	4	10	7	18	3
Do	Fifth apterous	4	10	8	15.5	2

The results obtained with *Rhopalosiphum persicae* were similar to those obtained with *Macrosiphum solanifolii*, especially in regard to the increased number of infections obtained with mature aphids over those resulting from the transfers of the immature stages. The length of the incubation period of the disease is also decreased where the older aphids are used. No marked differences could be observed in the ability of the two species to produce infections.

ABILITY OF APHIDS TO CARRY INFECTION TO MORE THAN ONE HEALTHY PLANT

A series of experiments were started to determine whether an aphid can carry the virus of the disease to more than one healthy plant after leaving the original diseased plant. Three series of inoculations were made. In the first series the insects were allowed to remain on the plants for 24 hours; in the second, 14 hours; and in the third, 2 hours. The diseased plants from which the aphids were obtained were carefully checked out by means of prick inoculations with virus, and positive proof of their being affected with the disease was thus obtained. The healthy plants used in the inoculations were 4 weeks old at the time the aphids were placed on them. Three or four plants were allowed to grow in 4-inch pots and the aphids were placed on these for each period. This gave a record of several plants in each series. The results of this experiment are given in Table VII. In comparing the results obtained with the transfers for the several periods it will be seen that where the insects remained on the plants for 24 hours the incubation periods were

shorter than when they remained on the plants for either 2 or 14 hours. There was apparently no greater number of infections obtained in the 24-hour period than in either of the others. These data prove that aphids have the ability to transmit spinach-blight to several plants after leaving a diseased host. In this manner alate forms flying from one healthy plant to another after leaving the blighted spinach might infect a large number of plants within a comparatively short time, and will perhaps explain the sudden and widespread appearance of blight which usually occurs after an outbreak of aphids, when many alate individuals are produced.

TABLE VII.—*Ability of aphids to carry infections to more than one healthy plant*

Plant No.	Length of time aphids remained on plants.	Number of plants.	Number of plants infected.	Average length of incubation period.	Number of insects per plant.	Number of plants remaining healthy.
	Hours.			*Days.*		
1	24	4	4	15	2	1
2	24	4	3	17	2	1
3	24	4	2	25	2	2
4	24	4	1	34	2	3
5	24	4	2	30	2	2
1	14	3	3	19	2	0
2	14	4	2	21	2	2
3	14	3	1	28	2	3
4	14	4	0	0	2	4
5	14	3	1	31	2	2
1	2	4	4	22	2	0
2	2	4	3	28	2	1
3	2	4	2	31	2	2
4	2	4	1	30	2	1
5	2	4	1	34	2	3

INOCULATIONS WITH THE JUICE OBTAINED BY CRUSHING APHIDS COLLECTED ON BLIGHTED SPINACH

As a check on the data collected in other experiments—namely, that the juice of crushed aphids from blighted plants is virulent—the following series was started. Several healthy plants were inoculated with the virus by means of needle pricks. When the plants had developed typical symptoms of blight, a large number of *Macrosiphum solanifolii* and *Rhopalosiphum persicae* were placed upon them and allowed to feed for a period of 20 days. A sufficient number of adults were collected from these plants and crushed, about 1 c. c. of juice being thus obtained. A series of healthy plants 28 days old was inoculated with this juice. Of 42 plants inoculated with juice of crushed *M. solanifolii*, 27 became infected, positive symptoms appearing in an average time of 23.1 days. Thirty-eight plants were inoculated with the juice of crushed *R. persicae*,

and 21 infections were produced. The symptoms appeared in an average time of 26.4 days. From these data it is evident that the juice obtained by crushing virus-bearing aphids is virulent.

TRANSFERS OF STRAINS OF APHIDS OBTAINED FROM OTHER STATES IN COMPARISON WITH TRANSFERS OF LOCAL APHIDS WHICH WERE SUPPOSED NOT TO BE CARRYING THE SPINACH-BLIGHT VIRUS

In some of the earlier experiments infections were produced when aphids which were supposedly not virus carriers were transferred to healthy plants. These led to the following experiment the object of which was to determine, so far as possible, the conditions which cause aphids to become virus carriers. In order to accomplish this, it was necessary to obtain strains of aphids from localities where, so far as is known, spinach-blight does not occur. Through the kindness of Dr. W. E. Hinds, Entomologist, Alabama Agricultural Experiment Station, a supply of *Macrosiphum solanifolii* on lettuce was obtained from Auburn, Ala. From Mr. Thomas H. Jones, Entomological Assistant, Truck Crop Insect Investigations, United States Bureau of Entomology, was obtained a large supply of *Rhopalosiphum persicae* collected on peppers in a greenhouse at Baton Rouge, La. Prof. C. P. Gillette and Mr. L. C. Bragg, of the Colorado Experiment Station, furnished us with a splendid lot of eggs of *R. persicae* on peach twigs from Fort Collins, Col. Prof. J. R. Watson, Entomologist, Florida Agricultural Experiment Station, furnished several live individuals of *R. persicae*, among other species collected from *Hibiscus sabdadriffa*.

As the various lots of aphids were received, they were placed, with the exception of a few individuals used immediately for experimental purposes, in cages on lettuce, eggplants, and healthy spinach. The aphids were allowed to feed and reproduce for several generations on the food plants mentioned until many thousand individuals of each strain were thus obtained. The eggs of *Rhopalosiphum persicae* were hatching at the time they arrived. The young stem mothers remained on the peach twigs until three generations of offspring were produced. The fourth generation was transferred to lettuce and healthy spinach. In this manner were obtained a sufficient number of aphids from widely separated regions, for use in comparison with local aphids collected in the vicinity of Norfolk, Va. Collections of local adult *Macrosiphum solanifolii* and *R. persicae* were made from diseased plants in the field. These were placed in cages on lettuce seedlings where they were allowed to feed and reproduce for five days. At the end of this period the adults were removed and destroyed. Some of the offspring were placed on other lettuce seedlings, some on eggplants, and the remainder on healthy spinach seedlings grown under insect-free conditions. The third week after the local aphids had been placed on the spinach seedlings some of these plants showed evidence of being diseased. Evidently they became

infected through the agency of the aphids placed upon them. The insects were confined on these plants for about five weeks during which time approximately four generations of first-born young were produced. This gave a large supply of supposedly nonvirus-carrying local aphids for use in making the transfers to healthy plants, and these insects and their offspring are referred to hereafter as the "Norfolk, Va., strain." The mean temperature in the cages in which the aphids were confined was 76° F., with a relative humidity varying from 55 to 95 per cent. The results of the transfers are given in Table VIII.

TABLE VIII.—*Transfers of strains of supposedly non-virus-bearing aphids obtained from various States in comparison with the transfers of the local (Norfolk, Va.) supposedly non-virus-bearing strain*

Species used.	Source of insect.	Previous food plant of insect.	Number of plants inoculated.	Length of time insects were on plant.	Number of plants infected.	Average length of incubation.	Number of insects placed on each plant.
				Hours.		*Days.*	
Macrosiphum solanifolii.	Norfolk, Va.....	Lettuce..	76	48	2	28	5
Rhopalosiphum persicae.	.....do.........	...do.....	52	48	3	27.3	5
Macrosiphum solanifolii.	.....do.........	Eggplant	24	48	2	31	2
Rhopalosiphum persicae.	.....do.........	...do.....	27	48	1	34	2
Macrosiphum solanifolii.	.....do.........	Spinach .	[a] 500	48	39	28.4	[a] 2
Rhopalosiphum persicae.	.....do.........	...do.....	[a] 500	48	27	32.1	[a] 2
Macrosiphum solanifolii.	Auburn, Ala...	Lettuce .	100	48	0	......	2
Do..............	.....do.........	Spinach .	100	48	0	......	2
Do..............	.....do.........	Eggplant	20	48	0	......	2
Rhopalosiphum persicae.	Baton Rouge, La.	Pepper...	50	48	0	......	2
Do..............	.....do.........	Spinach .	100	48	0	......	2
Do..............	Fort Collins, Colo.	Peach....	20	48	0	......	2
Do..............	.....do.........	Spinach .	50	48	0	......	2
Do..............	Gainesville, Fla.	...do.....	40	48	0	......	2

[a] Approximately.

To 76 healthy spinach plants were transferred 400 of *Macrosiphum solanifolii* (Norfolk, Va., strain) which had previously been feeding on lettuce. The insects remained on the plants for 48 hours. Two of the spinach seedlings developed positive symptoms of blight in an average time of 28 days. About 250 individuals of *Rhopalosiphum persicae* (Norfolk, Va., strain) which had previously been feeding on lettuce were transferred to 52 healthy spinach seedlings, on which they were allowed to feed for 48 hours. Three of the spinach plants developed symptoms of blight in an average time of 27.3 days. Fifty of *Macrosiphum solanifolii* (Nor-

folk, Va., strain) were transferred from eggplant to 24 healthy spinach seedlings. Two of the plants developed positive symptoms of blight in 30 and 33 days, respectively. Fifty of *Rhopalosiphum persicae* (Norfolk, Va., strain) which had been feeding on eggplant were transferred to 27 healthy spinach seedlings. One infection resulted, and the first positive symptoms developed on the thirty-fourth day.

In one of the large field cages seed was sown broadcast, and about 500 healthy plants were thus obtained. One thousand individuals of *Macrosiphum solanifolii* (Norfolk, Va., strain) which had previously been feeding on spinach were transferred to the plants in this cage. At the end of of the 48-hour period that the aphids were allowed to remain on the plants, the cages were fumigated on three consecutive days with nicotine. In this way all the aphids were destroyed. Thirty-nine cases of blight developed on the plants in an average time of 28.4 days. About 1,000 individuals of *Rhopalosiphum persicae* (Norfolk, Va., strain) were transferred to a second large field cage in which were growing approximately 500 spinach seedlings. After the aphids had remained on the plants for 48 hours, they in turn were destroyed by three successive fumigations. Twenty-seven of the plants in this cage became infected with blight. The first positive symptoms appeared in an average time of 32.1 days. Two hundred of *Macrosiphum solanifolii* (Auburn, Ala., strain) from healthy spinach were transferred to a cage containing 100 healthy spinach seedlings. No infections were obtained. Forty of *Macrosiphum solanifolii* (Auburn, Ala., strain) were transferred from eggplant to 20 healthy spinach seedlings. No infections were obtained. In a similar manner the following transfers were made. The insects remained on the plants for 48 hours in each case before they were destroyed. One hundred of *Rhopalosiphum persicae* (Baton Rouge, La., strain) from peppers, were transferred to 50 healthy spinach seedlings. Two hundred of *Rhopalosiphum persicae* (Baton Rouge, La., strain) were transferred from healthy spinach to healthy spinach seedlings. Forty of *Rhopalosiphum persicae* (Fort Collins, Colo., strain) were transferred from peach to 20 healthy spinach seedlings. . One hundred of *Rhopalosiphum persicae* (Fort Collins, Colo., strain) were transferred from healthy spinach to 50 healthy spinach seedlings. Eighty of *Rhopalosiphum persicae* (Gainesville, Fla., strain) were transferred from healthy spinach to 40 healthy seedlings. No infections were obtained as a result of any of the above transfers, and the seedling plants remained healthy in every case until the close of the experiments.

A series of healthy spinach plants were inoculated with the juice of the lettuce, eggplant, spinach, and pepper plants, upon which the aphids had been feeding previous to their transference to the healthy spinach seedlings in the experiment. No infections resulted from these inoculations, except from the inoculations with the juice of the spinach plants on which the Norfolk strain had been feeding, in which case 2 positive infections resulted from 10 inoculations.

A series of inoculations (see Table IX) were made, supplementing the transfers of the live insects. Aphids from the same sources as those used in the transfers were crushed and the juice thus obtained was inoculated into the tissues of the healthy plants by means of needle pricks. Fifty healthy plants were inoculated with the juice of *Macrosiphum solanifolii* (Norfolk, Va., strain) which had previously been feeding on lettuce. Four plants became infected, and symptoms of blight developed in an average time of 29.2 days. Fifty plants were inoculated with the juice of *M. solanifolii* (Norfolk, Va., strain) which had previously been feeding on spinach. Eight plants developed symptoms of blight in an average time of 31.6 days. Fifty plants were inoculated with the juice of *R. persicae* (Norfolk strain) which had previously been feeding on lettuce. One plant became infected. The first positive symptoms of the disease appeared in 36 days. Fifty plants were inoculated with the juice of *R. persicae* (Norfolk strain) which had previously been feeding on spinach. Four plants became infected in 31.9 days. In a similar manner healthy spinach was inoculated with the juice of *M. solanifolii* (Auburn, Ala., strain) from lettuce and healthy spinach. Healthy spinach was inoculated with the juice of *R. persicae* (Baton Rouge, La., strain) from pepper, healthy spinach, and lettuce. The juice obtained from crushed *R. persicae* (Fort Collins, Colo., strain) which had been feeding on peach and healthy spinach was inoculated into healthy spinach seedlings. All of these inoculations gave negative results, the plants remaining healthy until the experiment was closed, two months after the inoculations had been made.

TABLE IX.—*Results of inoculations with the juice of crushed, supposedly non-virus-bearing aphids*

Species used	Source of insect.	Previous food plants of insect.	Number of plants inoculated with the juice of crushed, supposedly non-virus-bearing aphids.	Number of plants infected.	Average length of incubation period.
					Days.
Macrosiphum solanifolii	Norfolk, Va.	Lettuce	50	4	29.2
Do	do	Spinach	50	8	31.6
Rhopalosiphum persicae	do	Lettuce	50	1	36
Do	do	Spinach	50	4	31.9
M. solanifolii	Auburn, Ala.	Lettuce	25	All healthy	
Do	do	Spinach	25	do	
R. persicae	Baton Rouge, La.	Pepper	25	do	
Do	do	Spinach	25	do	
Do	do	Lettuce	25	do	
Do	Fort Collins, Colo.	Peach	10	do	
Do	do	Spinach	25	do	

It is evident from the preceding data relative to aphids which have fed on blighted plants that a small percentage of their offspring, although these may have have been born and reared on plants other than spinach, may produce infections of blight when they are transferred to healthy spinach plants. Inoculations made with the juices of lettuce, eggplant, and supposedly healthy spinach produced no infections; hence, it is unlikely that either the lettuce or the eggplant served as alternate hosts for the inciting factor of the disease. The possibility that the blighted condition was due to a mechanical or other stimulus produced directly by the insect was disproved by the fact that aphids from the regions mentioned, where as yet the occurrence of spinach-blight has not been reported, were incapable of producing infections on healthy spinach unless they had fed on a diseased plant previous to their transference to a healthy plant. It was also found that the juice of Norfolk aphids, although born and reared on plants other than spinach, occasionally produced infections of blight when inoculated into healthy plants. The percentage of infection obtained by transferring the local strain of aphids from lettuce, eggplant, or peppers to healthy spinach plants was small. The transfers of local aphids from supposedly healthy spinach to known healthy spinach resulted in a larger number of infections than in cases where the aphids were transferred from lettuce or eggplant to healthy spinach. As it subsequently appeared, these supposedly healthy plants were diseased at the time the transfers were made from them to the known healthy plants. They became infected in all probability through the agency of the original aphids transferred from the lettuce seedlings. The plants were small and the aphids were numerous; therefore it was difficult to distinguish between the early visible symptoms of the disease and the somewhat yellowish condition caused by the attacks of numerous aphids. Subsequent inoculations proved that the juice taken from these plants was virulent; hence, it was a simple matter for infections to be carried from these to other plants when the transfers of insects were made. Inoculations made with the juices of lettuce, eggplant, and peppers used as food for the aphids before their transference to healthy spinach gave no indications of any infections.

In the light of these findings—namely, (1) that the offspring of local virus-bearing aphids, although born and reared on plants other than spinach, are capable under certain conditions of producing infections of blight in healthy spinach plants to which they have been transferred; (2) that the juices of the plants other than spinach upon which these aphids were reared were nonvirulent; (3) that aphids of foreign strains are incapable of producing the blight on healthy spinach, unless they have first fed on diseased plants—the assumption must be taken that, whatever the entity is which caused the pathological transformations in the growth and development of normal spinach plants, it must be in some manner transmitted from the parent aphids to their offspring, as the offspring in turn may cause infections in healthy plants upon which they feed.

LENGTH OF TIME NON-VIRUS-BEARING APHIDS MUST REMAIN ON DISEASED PLANTS BEFORE THEY BECOME VIRUS BEARERS

As it was found that the offspring of aphids from Alabama, Louisiana, Colorado, and Florida did not produce blight infections when reared on lettuce, eggplant, or healthy spinach unless they had first fed on a blighted spinach plant, the following experiments were performed to determine the length of time that these aphids must remain on a diseased plant before they become virus bearers. Offspring of *Macrosiphum solanifolii* from Alabama and *Rhopalosiphum persicae* from Louisiana were placed on blighted spinach plants. The disease had been produced by means of needle-prick inoculations and the pathogenicity had been proved before the aphids were transferred to them. The results are presented in Table X.

TABLE X.—*Length of time non-virus-bearing aphids must remain on diseased plants before they become virus carriers*

Species.	Length of time aphids remained on blighted plants.	Number of plants inoculated.	Number of plants infected.	Average period of incubation.
				Days.
Macrosiphum solanifolii (Alabama)	48 hours	10	9	18.1
Do	24 hours	10	8	17.2
Do	14 hours	10	10	18.7
Do	2 hours	10	6	21.3
Do	10 minutes	10	2	24
Control	48 hours	10	....	....
Rhopalosiphum persicae (Louisiana)	24 hours	10	7	17
Do	14 hours	10	6	17.5
Do	2 hours	10	4	20
Do	10 minutes	10	....	....
Control	48 hours	10	....	....

After the aphids had remained on the diseased plants for 10 minutes, 30 of *Macrosiphum solanifolii* (Auburn, Ala., strain) were removed and placed on ten 22-day-old spinach seedlings. The insects remained on the plants for 24 hours and were then killed by fumigation. Of the 10 plants thus inoculated 2 became infected, the positive symptoms appearing in an average time of 24 days. In a similar manner 10 healthy spinach seedlings were inoculated by transferring to them 30 of *Rhopalosiphum persicae* (Louisiana strain) which had previously been allowed to feed on the diseased plants for 10 minutes. No infections occurred. Thirty of *M. solanifolii* were removed after feeding on diseased plants for a period of two hours and placed on 10 healthy spinach seedlings. Six infections resulted, the positive symptoms appearing in an average time of 21.3 days. Ten plants were inoculated with *R. persicae* (Louisiana strain) which had been feeding on a diseased plant for two hours. Four plants became infected in an average time of 20 days. All of the 10 plants

inoculated with *M. solanifolii* which had been feeding on a diseased plant for a period of 14 hours were infected, the positive symptoms appearing in an average time of 18.7 days. Thirty of *R. persicae* which had been feeding on the diseased plant for 14 hours were transferred to 10 healthy plants and produced 6 infections. The symptoms appeared in an average time of 17.5 days. Similarly 30 of *M. solanifolii* which had been feeding on the diseased plant for 24 hours and 30 which had been feeding on the same plant for 48 hours were transferred to two lots of 10 healthy seedlings each. The aphids which fed on the diseased plants for 24 hours, produced 8 infections. The symptoms appeared in an average time of 17.2 days. The aphids which were allowed to remain on the plants for 48 hours produced 9 infections when transferred to the 10 healthy spinach seedlings. Positive symptoms appeared in the average time of 18.1 days. Thirty of *R. persicae* allowed to remain on the diseased plants for 24 hours, when transferred to 10 healthy spinach seedlings, produced 7 infections. The symptoms appeared in an average time of 17 days. As a check on the foregoing experiments, 30 of *M. solanifolii* and 30 of *R. persicae* were allowed to feed on healthy spinach plants for 48 hours and were then transferred to two lots of 10 plants each, on which they remained for 24 hours. No infections resulted.

The foregoing data indicate that a few aphids become virus bearers when they remain on diseased plants for 10 minutes. At the end of 14 hours the individuals of *M. solanifolii* reached the maximum in their capacity to transmit the virus, whereas individuals of *R. persicae* of the 24-hour group produced one more infection than did those which had been on the plants for only 14 hours. Probably under field conditions, where the insects are undisturbed, feeding takes place more readily than when they are more or less excited from artificial transfers, and they become virus bearers in much less time than experiments would indicate. According to findings reported elsewhere, when virus-bearing aphids are transferred to healthy plants, there is no appreciable relationship between the number of infections produced and the length of time the aphids remain on the healthy plants, although in the present instance there is a distinct relationship between the time the aphids which have not previously been associated with blighted plants remained on the diseased plant and the number of infections they subsequently produced. The longer the aphids remain on the diseased plants before being transferred, the shorter the time until visible symptoms of blight appeared on the inoculated seedlings.

DO IMMATURE APHIDS FROM DISEASED PLANTS BECOME NON-VIRUS-BEARERS AS A RESULT OF MOLTING?

A number of *Macrosiphum solanifolii* and *Rhopalosiphum persicae* in the fourth instar were collected in the field from a diseased plant and placed in individual sterile vials for a period of 24 hours. During this time they

were given no food. The individuals which had molted and become adults in the vials were transferred to healthy plants. Forty of *M. solanifolii* were placed on 20 healthy spinach plants on which they were allowed to remain for 48 hours. Six of the plants developed positive symptoms of the disease in an average time of 24.3 days. Forty of *R. persicae* were transferred to 20 spinach plants for a period of 48 hours. Four plants became infected and the symptoms appeared in an average time of 25.2 days. From these results it appears that when molting occurs after an aphid has left a diseased plant the insect may even then be able to produce infections of blight in healthy plants upon which it feeds, and indicates that the virus is transmitted in some other manner than on the external appendages of the insect's body.

ABILITY OF OFFSPRING OF VIRUS-BEARING APHIDS TO CARRY INFECTION

A series of healthy plants were inoculated by transferring to them at the time of birth the offspring of virus-bearing aphids. The young aphids had neither been allowed to feed previous to their transference to healthy plants; nor had they in any way come in direct contact with spinach affected with the disease. A number of adult female virus-bearing aphids were transferred directly to healthy spinach plants. The offspring of these females were transferred to the experimental plants as soon as they were born. Fifty of the immature *Macrosiphum solanifolii* were transferred to 25 healthy spinach plants and allowed to remain on them for a period of four days. They were then destroyed by fumigation. One plant became infected, and positive symptoms of blight appeared on the twenty-eighth day. As the young aphids had not taken food previous to their transference nor had any association with diseased plants before being placed on the healthy seedlings, the infections obtained of blight indicated that the young became associated with the infectious entity previous to their birth.

TRANSMISSION OF THE INFECTIOUS ENTITY OF BLIGHT BY ADULT VIRUS-BEARING APHIDS TO THEIR OFFSPRING

The results obtained in the earlier experiments on the insect transmission of spinach-blight indicated the possibility that the inciting factor of the disease was transmissible by a parent aphid to its offspring, thereby rendering the latter capable of producing infections in healthy spinach, although they had not previously fed on a diseased plant. The following experiments were performed to obtain data relative to this point. Both species of aphids were used and consisted of the Virginia, Alabama, and Louisiana strains previously discussed. The strains were confined in separate cages and were pure. On the same day the various lots of aphids were placed on fourth-stage-diseased plants in which the disease had been produced by virus inoculations made 42 days previously. The

adult aphids fed on the spinach for 7 days and were then retransferred to lettuce. On these plants they remained for 3 days, or until about 200 first-generation young had been born to each lot. The adults were removed, some being transferred to healthy plants for 48 hours. These were termed the "original parent aphids." When the offspring reached maturity and had produced between 200 and 300 young, the first-generation adults were removed and, as before, some were transferred to healthy plants. In like manner, as the second, third, and fourth generations matured, they were transferred to series of healthy spinach plants. Thus, inoculations were obtained from four generations representing three strains and two species of aphids. Two separate tests were conducted with each species: one in the insectary on potted plants and another in the field cages on plants grown under outdoor conditions. Five insects were placed on each plant and 10, 20, or 25 plants were used with each generation in each series. The results of this experiment are given in Table XI.

TABLE XI.—*Transmission of the infectious entity of spinach-blight by virus-bearing aphids to their offspring*

Species used.	Generation.	Number of insects per plant.	Number of plants inoculated.	Number of plants infected.	Average length of incubation period.
GREENHOUSE SERIES:					*Days.*
Macrosiphum solanifolii (Norfolk, Va., strain).	Original parent aphids from diseased plants.	5	25	23	18. 1
M. solanifolii	First	5	25	8	24. 2
Do	Second	5	25	2	33
Do	Third	5	25	2	34
Do	Fourth	5	25	1	37
OUTDOOR SERIES:					
M. solanifolii	Original	5	10	10	22
Do	First	5	10	2	37
Do	Second	5	10	3	34. 3
Do	Third	5	10	1	39
Do	Fourth	5	10	0	0
GREENHOUSE SERIES:					
Rhopalosiphum persicae (Norfolk, Va., strain).	Original virus-bearing aphids.	5	25	21	17. 4
Do	First	5	25	4	24. 2
Do	Second	5	25	2	26
Do	Third	5	25	1	24
Do	Fourth	5	25	0	0
OUTDOOR SERIES:					
R. persicae	Original	5	25	18	21. 3
Do	First	5	25	2	28
Do	Second	5	25	1	37
Do	Third	5	25	2	32
Do	Fourth	5	25	1	35
GREENHOUSE SERIES:					
M. solanifolii Auburn, (Ala., strain).	Original	5	10	9	17
Do	First	5	10	2	24
Do	Second	5	10	Healthy.	.....
Do	Third	5	10	2	32
Do	Fourth	5	10	1	36

TABLE XI.—*Transmission of the infectious entity of spinach-blight by virus-bearing aphids to their offspring*—Continued.

Species used.	Generation.	Number of insects per plant.	Number of plants inoculated.	Number of plants infected.	Average length of incubation period.
OUTDOOR SERIES:					*Days.*
M. solanifolii (Auburn, Ala., strain).	Original	2	10	10	21.3
Do	First	2	10	Healthy.	......
Do	Second	2	10	2	35
Do	Third	2	10	2	38
Do	Fourth	2	10	....	......
GREENHOUSE SERIES:					
R. persicae (Baton Rouge, La., strain).	Original	5	20	19	19
Do	First	5	20	2	28
Do	Second	5	20	Healthy.	......
Do	Third	5	20	1	31
Do	Fourth	5	20	Healthy.	......
OUTDOOR SERIES:					
R. persicae (Baton Rouge, La., strain).	Original	5	10	8	24
Do	First	5	10	3	27
Do	Second	5	10	1	36
Do	Third	5	10	2	35
Do	Fourth	5	10	....	......

The original virus-bearing Norfolk strain of *Macrosiphum solanifolii* gave high percentages of infection both on the insectary and field plants. One hundred per cent of these plants became infected in the field cages; this was unusual, considering that the aphids had fed on lettuce for several days between the time they were taken from diseased and the time they were placed on the healthy spinach plants. Eight infections out of 25 inoculations, or 32 per cent, occurred on those inoculated in the insectary with the first-generation offspring born and reared on lettuce. This was the highest percentage of infection obtained in any of the experiments with the lettuce-fed offspring of virus-bearing aphids. In the field the aphids from the same lot produced 2 infections from 10 inoculations. These from the second generation gave 30 per cent of infection in the field and 8 per cent in the insectary experiments. The third generation produced 8 per cent of infection in the insectary and 19 per cent in the field, while the fourth generation produced 4 per cent of infection in the insectary and none in the field. There was a gradual increase in the length of the average incubation period of the disease, from 18.1 days with the original virus-bearing aphids to 37 days with the fourth-generation offspring.

The results of the transfers of the Norfolk strain of *Rhopalosiphum persicae* were similar to those of local *Macrosiphum solanifolii*. With *R. persicae*, however, smaller percentages of infection were obtained

in nearly every case. The fourth generation produced no infection in the insectary experiments, although in the field one infection was obtained. The incubation periods of the disease in every instance were shorter than in the *M. solanifolii* series. Both lots of plants were kept under similar conditions and treated alike, and the infectious entity which the insects carried was derived from the same source. Observations of the feeding habits of the two species reveal that individuals of *R. persicae* were less disturbed by transference and not as liable to leave the plants as *M. solanifolii*. The former also fed considerably more during the 48-hour period of inoculation than *M. solanifolii*. It is possible that these slight differences in the feeding habits of the species would result in the transmittal by *R. persicae* of the more active infection, thereby shortening the period of incubation.

Macrosiphum solanifolii (Alabama strain) produced infection with all but the second generation under insectary conditions and the first and fourth generations on the field plants. The reason for not obtaining infection with the first and second generations in the instances cited may be due to several causes. These can not be explained until more is learned concerning the relationships existing between the insects and the causal factor of spinach-blight. The incubation periods for this series correspond closely to those obtained with the Norfolk strain of *M. solanifolii*. The inoculations with *R. persicae* (Louisiana strain) produced about the same number of infections as did *M. solanifolii* (Alabama strain) with the exceptions that no infections were obtained with the second generation under insectary conditions or with the fourth generation either in the field or in the insectary. The incubation periods of the disease were similar with both species.

It has been shown that certain supposedly non-virus-bearing aphids, collected locally on plants other than spinach and their offspring for several generations may occasionally produce infections of blight when allowed to feed on healthy spinach. No infections were obtained in any case where the aphids from other States were placed on healthy spinach plants, unless they had previously fed on diseased plants. The offspring of virus-bearing aphids when transferred to healthy spinach in previous experiments have occasionally caused infections of blight. This ability on the part of the aphids is confirmed by the results obtained in this experiment. The preceding data indicate that the inciting factor of spinach-blight is transmissible by parent aphids to their offspring for several generations. How this is accomplished is not known and probably will not be fully understood until the nature of the inciting factor of this disease has been discovered. There are undoubtedly several factors which exert a controlling influence on the ability of aphids to transmit the disease, when the causal factor is inherited from ancestors which have fed on blighted plants. From the data collected on the conditions which limit this factor the following appear to be the more important: The con-

dition of the diseased plant at the time it was used as food by the original ancestors; the temperature and humidity which prevailed both during the period when the aphids were feeding on the diseased plant and when their descendants fed on the healthy plants; and the parts of the plants on which the aphids fed. It is of importance to note in this connection that many aphids, although they may have fed on a diseased plant, are not necessarily virus carriers. From the data collected in the greenhouse and insectary experiments it appears that not over 50 or 60 per cent of the virus-bearing aphids produced blight in the healthy plants to which they were transferred. Likewise, many plants show a temporary immunity to the disease. In several of the earlier series plants were inoculated with a known virus and remained healthy for 72 days, at which time they were reinoculated. Infections occurred, and positive symptoms of blight appeared 17 days later.

Since it is possible under certain conditions for aphids to transmit the causal factor of spinach-blight to their offspring, thereby enabling the latter to produce infections in healthy plants on which they feed, it is of interest to note that, while it indicated a closer association between a plant pathogene and its transmitter than has hitherto been known to exist; yet there occur among animal and human diseases several instances where the relationships between the definitive parasites and their transmitters are similar to those found with spinach-blight.

For many years it has been known that certain animal diseases are communicable by the transference of virus from diseased to healthy individuals. The viruses are usually highly infectious and, although filterable, are thought to contain elements of a parasitic nature which thrive and reproduce in one or more hosts. Many zoologists have considered that these ultramicroscopic, infectious entities are probably protozoans. Several of the more important human diseases belong to the group caused by filterable virus. Little is known concerning their nature, except in some cases the method of their transmission, their alternate hosts, and various points in their life history. Knowledge of the definitive organism is lacking in each case.

Recently it has been found that there is a group of plant diseases that are caused by filterable virus. Studies of certain of these diseases indicate them to be of parasitic origin, and from the nature of spinach-blight—namely, that it is caused by an infectious virus, and that no microscopic organism has been found associated with the disease—it constitutes another addition to this peculiar group of plant diseases.[1]

[1] Through the courtesy of Dr. L. A. Hawkins, of the Bureau of Plant Industry, the juice of diseased spinach plants which had been filtered through a Berkefeld filter and a porous clay cup was obtained for inoculation purposes. A limited number of inoculations were made, but unfavorable cultural conditions caused the inoculated plants to yield rather uncertain results; therefore it seems advisable to repeat these experiments under more favorable conditions before the results are published.

There are, then, two groups of diseases: One affecting animal life, the other affecting plants. The pathogens in the groups have several points of similarity. First, they are evidently parasitic; second, they are caused by an infectious virus; third, their manner of transmission from diseased to healthy hosts is usually through the agency of animal parasites infesting the hosts—that is, insects, mites, or ticks; fourth, students of both groups of diseases generally believe the definitive parasites to be ultramicroscopic organisms. Among animal diseases there are several which are transmitted by insects, mites, or ticks. Rocky Mountain spotted fever, a disease caused by an infectious virus is carried from diseased to healthy hosts by several species of ticks. Ricketts [1] found that not only was infection carried by the adult tick but a percentage of the offspring of the ticks from diseased animals inherited the ability to produce the disease. This case is similar to spinach-blight in which the causal factor of the disease is transmitted by the parent aphids to their offspring. The infectious entity of yellow fever has not been definitely proved to pass from adult mosquitoes to their offspring, yet the later experiments by Finlay [2] indicated the probability that the infectious entity was hereditary in certain species of Calopus.[3]

The well-known Texas fever of cattle was found by Smith and Kilbourne to be caused by a definite organism. The organism is transmitted by ticks on affected cattle to their offspring.

SUMMERING OF SPINACH-BLIGHT

ALTERNATE HOSTS

If the disease survives the summer on plants other than spinach, it is probably carried by aphids both to and from the other species of plants. Certain insects feed on spinach only in the spring and fall; hence, it is possible that it may be carried by one of these species instead of by those commonly inhabiting the spinach during the winter as well as in the spring and fall. The combined known food plant list of *Macrosiphum solanifolii* and *Rhopalosiphum persicae* comprises more than 100 species, representing a wide range of botanical groups, any of which might possibly be alternate host plants of the disease. So far as time would permit, those species closely allied botanically to spinach have been carried through series of inoculations the results of which were negative. Many species appeared to be affected with mosaic diseases, and these were used to inoculate healthy spinach plants, but without success in producing infectious blight. Only by a great amount of systematized work entailing thousands of inoculations can any definite proof be obtained relative to the question of alternate hosts of the inciting factor of spinach-

[1] RICKETTS, H. F. SPOTTED FEVER REPORT. no. 1/2. *In* 4th Bien. Rpt. State Bd. Health Mont. 1907–8, p. 87–191. 1908

[2] FINLAY, C. J. TRABAJOS SELECTOS (SELECTED PAPERS). 657 p. Habana, 1912.

[3] SMITH, Theobald, and KILBORNE, F. L. INVESTIGATIONS INTO THE NATURE, CAUSATION, AND PREVENTION OF SOUTHERN CATTLE FEVER. *In* U. S. Dept. Agr. Bur. Animal Indus. 8th/9th Ann. Rpt., 1891/92, p. 177–304, 10 pl. 1893.

blight. Thus far it has been impossible to give this phase of the problem more than the briefest consideration. Further data along this line are being accumulated as the experiments proceed.

RELATIONSHIP OF INSECTS TO THE SUMMERING OF THE DISEASE

As certain aphids have been found to possess the ability to transmit the causal factor of spinach-blight to their offspring, the question arises, Do aphids then serve as a means of carrying the disease over from the time the crop is harvested in the spring until the spinach is planted in the autumn? The evidence we have would indicate that succeeding generations from the original parent aphid from diseased spinach gradually become less infective and the percentage of virus-bearing offspring decreases with each generation, provided they do not have access to diseased food. The condition of the diseased plant at the time the parent aphids feed on it is an important determining factor in the transmission of blight by their offspring. Also, the numbers of the aphids are reduced to a minimum in July and August, which would mean that a very small percentage of those which have the earlier conditions entirely favorable would survive to produce offspring which eventually reach the spinach in the fall. When blight first appears in the autumn, it occurs usually as widely separated cases, from which infection is carried to surrounding plants by aphids. In fact, the first blighted plants to be found in the autumn are about as numerous as the aphids bearing virus by heredity might be expected to be. In the autumn of 1917, collections were made of numbers of both *Macrosiphum solanifolii* and *Rhopalosiphum persicae* from various cultivated plants and weeds and placed on healthy spinach seedlings for a few days. Positive results were obtained with 10 adults of *M. solanifolii* collected on the vines of sweet potatoes (*Ipomoea batatas*) on September 24, 1917, and with three adults of *R. persicae* collected from celery (*Apium graveolens*) on October 1. A number of *M. solanifolii* were placed on five spinach seedlings in the insectary, and two of them developed symptoms of blight on October 30. Similarly, on November 1, positive symptoms appeared on one of the two plants on which the individuals of *R. persicae* were allowed to feed. At the time the insects were collected, healthy spinach plants were inoculated with the juice of the plants from which the aphids had been collected. These inoculations gave negative results.

On June 4, 1917, in the potato field on farm A, which had been in spinach during the winter and early spring, a number of *Macrosiphum solanifolii* were collected on wild mustard plants growing near the center of the field where blight had been serious during the previous winter. These aphids were placed on pots of healthy spinach plants in the greenhouse; similar pots of plants were kept as controls. Seven days later some of the spinach plants showed doubtful symptoms of blight. Six days after this some of the plants developed the characteris-

tic symptoms of blight. These results indicate that *M. solanifolii* on the wild mustard plants were still virus bearers, although they or their parents had not fed on spinach for some time, as the spinach in this field had been cut early in March. Inoculations with juice of the mustard plants gave negative results.

INFECTIOUS ENTITY OF SPINACH-BLIGHT MAY BE CARRIED FROM SPRING TO FALL BY A DIRECT LINE OF APHIDS

On April 6, 1917, a blighted plant was obtained from farm B and brought to the greenhouse to use for inoculations. One adult of *Macrosiphum solanifolii* was removed from the blighted plant and placed on the larger of two spinach plants 74 days old, growing in a pot. A similar pot of plants served as a control. Both of the plants in the pot to which the aphid was transferred developed positive symptoms of blight, while the controls remained healthy.

On May 7, 1917, both of the inoculated plants were in advanced stages of blight. The direct descendants of the adult aphid, used for the inoculation on April 6, were abundant on these blighted plants. On May 7 some of these direct descendants were transferred from the blighted spinach plants to a small potted eggplant which had been grown in a large cage under insect-free conditions. This eggplant was then removed from the pot and transplanted to the soil in a field cage which was free from spinach. Several potato tubers were planted in the soil about the eggplant. On May 14 several Ruby King pepper plants which had been grown under insect-free conditions were transplanted to the above field cage, and to them were transferred young aphids from the eggplant. These aphids were kept in a field cage and allowed to reproduce and feed only on eggplant, pepper, and potato plants during the summer.

On August 9, 1917, a number of the above aphids in various stages of development were transferred to healthy spinach seedlings growing under insect-free conditions in another field cage. Similar seedlings in another field cage served as controls. On September 1 some of the plants to which the aphids had been transferred on August 9 were slightly mottled, while the control plants were of a normal green color. The aphids multiplied rapidly on the spinach plants in the field cage, and by October 1 the majority of the plants had died without showing any decided mottling. On October 6 new spinach seed grown in New York State was broadcasted in this cage and raked into the soil. A thick stand of spinach seedlings came up in six days. To these seedlings the aphids migrated from the few yellowish plants which still remained alive. On November 10 a few of the plants from the broadcasted seed had developed the mottled leaves characteristic of blight. On November 15 a considerable number of the seedling plants in this cage had yellow cotyledons, and the true leaves of many were distinctly mottled. At this time only one of the original plants growing in this cage remained

alive, and it had only a small whorl of mottled leaves at the center, the older leaves having degenerated, similar to those of blighted plants in the field. On November 15 one mottled leaf was removed from this remaining large plant, and with it a pot of several spinach seedlings growing in a greenhouse cage was inoculated by mashing the diseased tissues into the leaves of the seedling. Seedlings in a similar pot were mashed with a flamed needle to serve as controls. On November 27 some of the seedling plants in the pot inoculated on November 15 had developed the typical mottled leaves.

On November 15 two mottled spinach seedlings from the seed planted in the field cage on October 6 were removed from this cage, and with them one pot of healthy spinach seedlings growing in a greenhouse cage was inoculated by mashing the diseased tissues into the potted seedlings. A similar pot of seedlings was mashed with a flamed needle and served as a control. On November 27 mottled leaves had developed on the inoculated seedlings, but the control plants appeared healthy.

On August 9, when the aphids were transferred from the field cage in which they had summered to spinach seedlings in another field cage, some leaves from the potato and pepper plants on which they had summered were removed from the field cage, and potted spinach plants in a cage in the greenhouse were inoculated with them by mashing their tissues into the leaves of the spinach seedlings. These spinach seedlings were under observation until November 27, but no signs of mottled leaves developed, and the plants grew to a large size and had the dark-green color characteristic of healthy spinach plants. These results indicate that the pepper and potato plants on which the aphids had fed during the summer did not act as alternate hosts for the spinach-blight virus.

The record of the direct line of aphids kept on pepper, eggplant, and potato plants in a field cage during the summer and then transferred to spinach at about the time early spinach is planted in the field in the fall indicated that a direct line of aphids from a known virus-bearing parent may carry the infectious entity of spinach-blight over the summer even though they do not feed on spinach during that time. This record substantiates the other evidence which has been accumulated and points toward the probability that aphids are the important factor in the summering of the spinach-blight virus.

RELATION OF LEFT-OVER SPINACH PLANTS TO THE SUMMERING OF SPINACH-BLIGHT

It often happens that, when spinach fields are plowed after the late spring crop has been harvested, plants may be left growing along the edges of the field. On July 8, 1917, several diseased plants were found which had escaped the plow earlier in the season. Either species of aphids whose life averages between 30 and 40 days could pass the time elapsing

between the 1st of July and the time the early fall spinach is above the ground by four last-born generations; hence, in this case one of the factors hindering spring-to-fall transmission of the disease by aphids would be eliminated.

At the edge of a field on a farm near the Station one spinach plant left from the spring crop was observed on June 4, 1917. This plant had been left because it was in line with a cucumber row. It did not appear to have typical symptoms of blight, although some of the leaves were yellow. A number of *Macrosiphum solanifolii* were present on this plant. One leaf-bearing aphid was removed and brought to the greenhouse, where the aphids were placed on a pot of spinach seedlings. Another pot of seedlings was inoculated by mashing the tissues from the field plant into the leaves of the healthy seedlings. Similar pots of plants were pricked with a flamed needle to serve as controls. Fourteen days later, in the pot to which the aphids had been transferred, there were several plants with the mottled leaves characteristic of blight. Twenty-six days after inoculation three plants having characteristic symptoms of blight were observed in the pot of seedlings which had been inoculated with the leaf of the field plant from which the aphids were removed. These results indicate that the few plants which are allowed to remain in the fields after the regular crop has been harvested are the collecting places for numerous aphids, thus making the possibilities great for such plants becoming diseased with spinach-blight, and serving to carry the disease well into the summer after the regular crop has been harvested. It is known that this field plant grew for some time after aphids had been transferred from it to the greenhouse. A rank growth of weeds eventually surrounded this plant, so that it was impossible to determine how long it remained alive and served as a source of infection. The evidence which we have points toward the probability that aphids are instrumental in carrying the disease over the summer by their power to transmit the casual factor from parent to offspring for several generations. Probably this is not the only means by which spinach-blight may pass the summer period. As more is learned concerning the relationship between blight, insects, and plants other than spinach, doubtless other means may be discovered.

ABILITY OF OTHER INSECTS INFESTING SPINACH TO TRANSMIT SPINACH-BLIGHT

With the exception of the bean aphis, none of the species included are generally active during the winter months, and for these reasons are not liable to become important, from their agency as transmitters of blight virus. Experiments were performed in which most of the insects infesting spinach in this region were transferred from diseased to healthy plants. The insects remained on the plants in each case for 48 hours.

BEAN APHIS (*Aphis rumicis* Linnaeus).—Should this aphis become abundant at a time when blight is prevalent, it will undoubtedly be an important factor in the transmission of the same, but the species is generally abundant only during the summer months and is rarely found on spinach in this region. On Long Island, New York, it occurs on spinach grown for seed. Specimens received on July 12, 1917, on blighted spinach plants from Long Island were placed on healthy spinach seedlings. Infections occurred, and positive symptoms of blight appeared on August 2, thus proving the ability of the species to transmit the disease, as well as giving a record of the occurrence of blight on Long Island.

TARNISHED PLANT BUG (*Lygus pratenis* Linnaeus).—Two infections were obtained when specimens from blighted plants were transferred to 10 healthy spinach seedlings. This species occurs abundantly on spinach growing after March 20. As late-grown spinach is usually not harvested until April it is possible that this insect may be partially responsible for outbreaks of the disease at this time. In the autumn occasional specimens of *L. pratensis* have been collected on spinach as late as November 25, but our records would not indicate that they are sufficiently abundant to be of importance in causing early infections of blight.

SOUTHERN CORN ROOTWORM (ADULT) (*Diabrotica 12-punctata* Olivier).—This insect occasionally feeds on spinach when other preferable food is scarce. No infections were obtained when individuals were transferred from diseased to healthy spinach plants.

GREEN PLANT BUG (*Nezara hilaris* Say).—This species has been collected on spinach in October and in April. Individuals which were known to have fed on a diseased plant were transferred to healthy plants and allowed to feed on them for 48 hours. No infections resulted from these transfers. A few individuals of *Euchistus servus* Say have been collected on spinach, and some from a diseased plant were transferred to known healthy plants and allowed to feed. They gave negative results so far as obtaining a transmission of blight was concerned.

Thrips tabaci Lindeman, *Smynthurus hortensis* Fitch, and *S. quadrimaculatus* Ryder may occur abundantly on spinach during the fall and spring, but no infections were obtained when these insects were transferred from diseased to healthy plants.

OCCURRENCE OF SPINACH-BLIGHT IN OTHER STATES

NEW YORK.—On July 14, 1917, diseased spinach plants from western New York were received. These showed the typical characteristics of blight. Virus was obtained and inoculated into 15 healthy spinach seedlings in a field cage. Ten healthy spinach seedlings were used as controls. On July 25 nine of the inoculated plants had developed symptoms of blight, and by August 1 eight were distinctly mottled, and the leaves malformed. Seven of the inoculated plants died shortly after

the inoculations had been made. No evidence of the disease appeared on any of the control plants. On August 6 virus was obtained from the blighted plants and used to inoculate several seedlings grown in a pot in the greenhouse under insect-free conditions. A similar pot of spinach seedlings was untreated and served as a control. On August 22 it was observed that the plants inoculated on August 6 had developed the characteristic mottled appearance of blight. All the control plants remained healthy. This gives definite proof that spinach-blight occurs in western New York as well as on Long Island.

OHIO.—Two boxes of diseased spinach plants of the Viroflay type were received from Ohio. These plants were in various stages of what appeared to be typical spinach-blight. In Ohio the disease is known by the name of "yellows" (Pl. 10, A).

Individuals of both *Macrosiphum solanifolii* and *Rhopalosiphum persicae* were present on the plants. Aphids were removed and placed on pots of Savoy spinach seedlings growing in the greenhouse. Similar plants were used as controls. Mottled leaves were removed from each of the plants from which the aphids were taken, and Savoy spinach seedlings were inoculated by mashing the mottled leaves into them. Ten days after inoculation it was observed that some of the potted seedlings were showing doubtful symptoms of blight. Six days later some of the inoculated plants had developed characteristic symptoms. The control plants remained healthy.

At the same time the inoculations were made in the greenhouse, plants in one of the field cages were inoculated by mashing the diseased tissues from the Ohio spinach into the leaves. A number of plants were thus inoculated, and a similar number were pricked with a flamed needle serving as controls. Sixteen days after inoculation some of the plants in the field cage had developed typical symptoms of blight, but the control plants remained healthy. Six days later one of the mottled leaves was removed from each of the two blighted plants in the field cage. Pots of spinach seedlings 16 days old were inoculated by mashing the diseased tissues into the cotyledons of the seedlings. A similar pot of plants served as a control. Eight days after inoculation 14 of the plants developed positive symptoms of blight, the control plants remaining healthy. Twelve days after observing positive symptoms of blight in the field cage one mottled leaf was removed from a blighted plant, and with it 16 plants 20 days old and 29 plants 11 days old were inoculated by mashing the diseased tissues into the cotyledon and the first true leaves. Eleven days after inoculation the majority of the plants in each of the two pots had developed mottled leaves, but the control plants remained healthy.

From these results it appears that the Ohio spinach is subject to the same disease which is present in the Norfolk section and that the disease is caused by the same virus which causes spinach-blight in Virginia. It is interesting to note that spinach-blight virus is evidently readily trans-

ferable from the Viroflay to the Savoy type, and vice versa, thus indicating that the same disease may develop in various sections where spinach is grown, regardless of the type of plants used.

SOIL TRANSMISSION OF SPINACH-BLIGHT

To determine whether spinach-blight is carried in the soil, blighted areas were selected in each of three fields on an adjoining farm. The blighted plants were removed from the areas in each field, and one flat was filled with soil from each area, care being exercised to take the soil from around and under where the blighted plants had developed. The three flats of soil were brought to the greenhouse and covered with cloth cages. A fourth flat of the same size was filled with steamed greenhouse soil to serve as a control. The following night the greenhouse was fumigated with tobacco to kill insects. Three days after bringing the flats to the greenhouse they were planted with four lots of spinach seed from different sources. The control flat was planted with similar seed. Previous to planting, the seed was soaked in 1 to 100 formaldehyde for a few minutes. The four lots of seed in each flat were separated from one another by thin board partitions. For a period of 75 days these flats were left in the greenhouse. No signs of blight developed in any of the plants. The plants in the flats of field soil were as healthy as those from the same seed grown in steamed soil. Therefore it would appear that spinach-blight is not carried in the soil.

To insure that neither the transfer of soil from the field to the greenhouse nor the modified conditions of the greenhouse was responsible for the failure of blight to develop on the plants grown in the various lots of soil, this experiment in somewhat different form was duplicated in the field on an adjoining farm. A bed of spinach which had a number of blighted areas in it was used. Four areas were selected where uniform outbreaks of blight had developed. The blighted plants, including the roots, were removed from the soil, and all traces of vegetation which might harbor insects were removed from the first three areas selected. In the fourth area the blighted plants were left exactly as they grew in the field. A trench the size of a cage approximately 2 by 3 feet in area was dug, the cage set therein, and the soil well banked about the outside in order to insure that no insects should enter from below. After the four cages had been placed, the soil within them was treated as follows:

In cage 1 the soil was loosened and planted to two lots of spinach seed which had been disinfected in 1 to 100 formaldehyde for a few minutes before planting. Four days later, before the spinach plants were up, the soil in this cage was soaked with a 40 per cent nicotine-sulphate solution (1 to 100) in order to kill any insects. Cage 2 was planted with spinach seed which had been disinfected with 1 to 100 formaldehyde. The soil in this cage was left in the same condition as after it had grown blighted plants, no attempt being made to free it of insects. In cage 3 the soil was treated in

a manner similar to that in cage 1 and was planted with two different lots of spinach seed. In cage 4 seven of the original field plants were present. Of this number one was apparently healthy while the others were in various stages of blight. Care was exercised not to disturb the aphids which were feeding on the plants. Four commercial lots of spinach seed were planted in this cage, care being taken to include lots planted in each of the other cages. No attempt was made to free this cage of aphids or other insects by the use of nicotine solution. Twelve days after planting it was observed that spinach seedlings in each cage were growing vigorously. Twenty-four days after planting, the young plants in all four cages appeared healthy, but aphids were observed on the seedlings immediately surrounding the mature plants in cage 4, while none were present in the three other cages. Ten days later the seedlings in cages 1, 2, and 3 all appeared healthy, while numerous seedlings in cage 4 bore wrinkled, much mottled leaves characteristic of blight. Four days later seedlings in cage 4 were dwarfed and mottled, and the cotyledon leaves were yellow and were dying. Many aphids were observed on the seedlings in cage 4, but none were seen on any of the seedlings in cages 1, 2, and 3. Seven days later all but a very few of the plants in cage 4 were dwarfed and mottled, while the plants in cages 1, 2, and 3 appeared healthy. Nine days later photographs were taken to show the characteristic blighted appearance in cage 4 on that date (Pl. 10, B) as compared with the healthy plants in cage 2 (Pl. 11, A). Healthy greenhouse plants were inoculated with the tissues of diseased seedlings from cage 4. These eventually developed typical symptoms of blight. Similar plants used as controls remained healthy. The plants in all four cages continued to grow until late in May, when the aphids became so abundant in cage 4 that practically all of the plants were killed. During the 44-day period after planting the seed the plants in cages 1, 2, and 3 grew more rapidly and became much larger than those in cage 4, showing no symptoms of blight. About six weeks after planting, the cloth door of cage 3 was found open, probably owing to a severe wind storm the night before. About 500 plants were removed from cage 3 at this time in an attempt to find aphids or blighted plants. Neither were found; so the remaining 500 plants or more were left in the cage. The experiments in cages 1 and 2 continued until the end of the season without the plants in either cage showing signs of blight. Some time after the door of cage 3 was found open, aphids were observed on the plants within this cage, and before the end of the season spinach-blight had developed on some of the plants. The results obtained in these four cages substantiate those obtained in the greenhouse and indicate that spinach-blight is not carried in the soil, but is carried from plant to plant by insects or mechanical means.

SEED TRANSMISSION OF SPINACH-BLIGHT

The occurrence of spinach-blight from time to time in commercial fields, and especially in new land which had never been planted to spinach before, suggested the possibility of seed transmission of this disease. During the period that the various experiments on the transmission of this disease have been conducted many thousands of spinach plants have been grown, some even to maturity in both greenhouse and field cages. In no case has there been any evidence that blight was transmitted through seed. When it is considered that the various seed strains used for this work have been obtained from practically all parts of the United States and Europe where spinach seed is grown, it would appear that the weight of data is against the idea of seed transmission of spinach-blight.

During the spring of 1917 seed was collected from over a hundred blighted plants in various stages of the disease. Unfortunate circumstances following the harvesting of this seed made it impossible to get immediately as many data as were desired relative to the transmission of spinach-blight by these seed; therefore this phase of the problem is not complete. The growing of spinach plants from the seed of these diseased plants is being conducted on a rather extensive scale, both in the field and in the greenhouse.

POSSIBLE MEASURES OF CONTROL

CONTROL OF INSECT CARRIERS OF THE INFECTIOUS ENTITY

Probably the most effective and immediate control of spinach-blight can be obtained by destroying or otherwise eliminating the transmitters of the infectious entity of the disease. As aphids are usually the most abundant insects on spinach in this region during the time blight is present, efforts are being made at the present time to control them. During the winter parasites of the aphids are not as effective in holding them in check as they are during warmer periods. Also the predacious enemies of the aphids, particularly the ladybird beetles *Hippodamia convergens* Guérin and *Megilla maculata* De Geer, are hibernating and are of little benefit in reducing outbreaks of aphids between November 1 and April 1; hence, except for temperature conditions, natural control of aphids can not be counted upon to relieve the situation at this time. Experiments have been conducted for several years on spraying spinach for the control of aphids. It was noticed in 1914 and again in 1916 that on sprayed plots where the most effective control of aphids was obtained there was much less blight than on the unsprayed plots where the aphids were allowed to feed undisturbed. Not until the autumn of 1917 was an arrangement devised whereby spinach plants could be effectively sprayed to kill the plant lice. The present indications are that blight can be materially reduced by the timely application of sprays for the control of aphids.

Extensive work along this line is now under way, and it is hoped that by another season the results will have borne out their present indications and methods devised which may be practically applied on a commercial scale.

EXPERIMENTS ON PRODUCTION OF BLIGHT-RESISTANT SPINACH

In the fall of 1916 a number of strains of the Savoy spinach were obtained from various parts of the United States and Europe. These seed were planted in separate beds on a piece of land about 1 acre in area, where in 1914 spinach-blight was so serious that not a barrel of marketable spinach was harvested. Blight developed again in 1915 and killed a large percentage of the plants before spring, but a few plants remained alive and produced seed. The seed from each plant was saved separately. In 1916 it was planted on the same land. Additional strains of commercial seed were again planted on this land, as was also the supply of seed from crosses of various types of spinach, including some importations from Asia, grown at Concord, Mass., by Mr. J. B. Norton, of the Bureau of Plant Industry. It was observed that the 1915 selections from commercial strains were superior both in type of plants and in disease resistance to the commercial strains used in 1916. It was also observed that the seed furnished by Mr. Norton showed greater resistance to blight than any of the other lots of seed (Pl. 11, A). Plants from the Massachusetts-grown seed varied widely in types, but a limited number of good Savoy plants were present. In the spring of 1917 seed was saved from the Savoy plants of the Massachusetts-grown strain, from the 1915 selections and from the commercial strains. During the fall of 1917 the various lots of selected seed were tested further, both on the experiment station farm and to a limited extent on a number of widely separated commercial fields. Although the results obtained thus far are encouraging, the nature of spinach-blight and its method of dissemination is such that it seems best to consider that the above experiments point to a possible means of control, rather than an immediate solution for the problem. Breeding experiments are being continued with the Savoy spinach and also with several types of spinach which are used commercially in other parts of the United States.

SUMMARY

(1) Spinach-blight has caused a greater annual loss to the trucking interests of eastern Virginia than any other single disease. It has been conservatively estimated that spinach-blight causes an annual loss of at least $200,000.

(2) Spinach-blight is a specific disease characterized by a mottling and malformation of the leaves and a decided stunting of growth. The diseased plants go through a number of characteristic stages and finally die. Diseased plants may occur in definite areas or they may be scattered over the field.

(3) Spinach-blight is distinguished from fungus diseases by the fact that there is no specific microscopic organism associated, and that the various fungi produce definite leafspots, while spinach-blight causes a gradual degeneration of the tissues.

(4) Opinions vary as to the time when spinach-blight first appeared in eastern Virginia. One grower reports it as serious at least 13 years ago.

(5) Mr. L. L. Harter, of the United States Department of Agriculture, worked on malnutrition diseases of various truck crops, and it is evident from his published reports that spinach-blight was included among these diseases. The early work on these diseases resulted in the use of better cultural methods.

(6) This blight has increased in seriousness from year to year. During the past 10 years the disease has spread until it is now present annually throughout eastern Virginia wherever spinach is grown commercially.

(7) For some years past it has been observed that spinach-blight became most serious within a short time after aphids were observed to be abundant on the plants. These observations led growers to suspect that the aphids were the direct cause of this disease.

(8) Some spinach growers were of the opinion that spinach-blight was due to poor soil drainage. Data relative to this point proved that the drainage within blighted areas was usually as good and often better than that in other parts of the field where the plants were healthy.

(9) Fertilizer experiments conducted in 1915 and 1916 proved that stable manure, lime, and commercial fertilizers applied to land where spinach-blight had been very serious the year previous had no direct influence on the development of the disease. Experiments with the substitution of fertilizer elements not commonly used, for those generally employed in making up commercial fertilizers had no effect in reducing the amount of blight.

(10) By a repetition of earlier work in an attempt to determine whether or not fungi or bacteria were associated with blighted plants, it was found that tissues of plants in early stages of the disease remained sterile on plates of nutrient media. Plants in more advanced stages of spinach-blight, where the tissues were breaking down, yielded numerous fungi and bacteria. When healthy spinach plants were inoculated with pure cultures of each of these organisms, no blight was produced.

(11) Inoculations made in the winter of 1915–16 with the juice of blighted spinach plants gave indications that the disease was of an infectious nature.

(12) In eastern Virginia spinach is grown in the fall, winter, and early spring. During this period two species of Aphididae, the potato aphis (*Macrosiphum solanifolii* Ashmead) and the spinach aphis (*Rhopalosiphum persicae* Sulzer) are the most abundant insects on spinach. Several other species of insects are found associated with spinach during

early fall and late spring, but thus far experiments have proved these of minor importance in connection with spinach-blight.

(13) Differences in the habits of the aphids, however, cause *Macrosiphum solanifolii* to be the more important agent in the dissemination of the disease. Both aphids infest many species of plants. Experiments to determine whether any of these may constitute alternate hosts for the inciting factor of spinach-blight are not completed.

(14) Direct transfers of virus-bearing aphids to healthy plants produced infections of spinach-blight.

(15) Inoculation with the juice of crushed virus-bearing aphids produced infections of blight.

(16) Transfers of aphids which had not previously fed on diseased material produced infections of blight in a few cases.

(17) Inoculations with the juice of lettuce, eggplant, peppers, and potato, used as food for the aphids, did not produce infections.

(18) Blighted plants collected from various local farms and from blighted areas and individual diseased plants proved to be due to similar virus in all cases.

(19) Virus-bearing aphids produced infections in healthy plants when allowed to feed on them for two minutes.

(20) The infectivity is greater with adult aphids than with those which are immature, and the incubation period of the disease produced by the adults is materially less than when the disease is produced by the immature forms.

(21) Aphids have the ability to carry infection to several healthy plants after leaving the diseased host.

(22) Supposedly non-virus-bearing aphids were found to cause infections of blight when transferred to healthy spinach. Aphids from Louisiana, Alabama, Florida, and Colorado when transferred to healthy spinach did not cause infections of spinach-blight unless they had previously been allowed to feed on diseased spinach. Inoculations with the juice of crushed aphids from other States yielded similar results.

(23) Non-virus-bearing aphids must remain on diseased plants for 5 minutes to 14 hours to become carriers of infection.

(24) Virus-bearing aphids do not lose their ability to transmit the causal entity of spinach-blight during the process of molting.

(25) Infections were obtained with the offspring of virus-bearing aphids which had not previously partaken of food.

(26) The infectious entity of spinach-blight was found to be transmitted by virus-bearing adult aphids to their offspring. It was also found that although aphids were reared on lettuce for four consecutive generations, yet a few of the fourth generation were virus bearers and produced infections when they were transferred to healthy spinach. These results show similarity to certain animal diseases caused by virus and transmitted by insects or ticks.

(27) Since it has been found that the causal factor of the disease may be hereditary with the aphids, this pointed to the possibility of its summering by this method. Experiments have shown that aphids collected on spinach plants left after the crop had been harvested may be virus bearers, as are also aphids collected from weeds growing later in the season in the same fields.

(28) Experiments with aphids from plants other than spinach during the fall produced spinach-blight in a limited number of cases. The direct offspring of a known virus-bearing aphid reared during the summer in a field cage on pepper and potato plants produced blight when they were transferred to spinach seedlings in August, or about the time early spinach is coming through the ground. Infections were obtained in a small number of cases with several other species of Hemiptera. These are probably not important as blight transmitters, as they do not occur abundantly at the time blight is prevalent.

(29) Spinach-blight has been found on Long Island and in western New York both on seed spinach and on scattering plants left from the canning crop.

(30) Blight was also found in Ohio on spinach grown by market gardeners.

(31) Experiments to date indicate that spinach-blight is not transmitted through the soil.

(32) From the data collected it is probable that spinach-blight is not transmitted by seed.

(33) The control of the aphids infesting spinach offers the most immediate possibilities for the control of spinach-blight.

(34) Experiments are under way for the breeding of blight-resistant seed, but these do not offer any immediate solution for the spinach-blight problem.

PLATE A

1.—A typical blighted Savoy spinach plant in the fifth stage of the disease. Note the difference in size and color of this plant and the healthy plant shown in figure 2. The plants were the same age and were drawn to the same scale. (Original.)

2.—A healthy Savoy spinach plant. Note the deep-green color, the tugidity, and larger size, which characterize the healthy plant. (Original.)

1

2

PLATE 1

A.—*a*, A spinach plant killed by blight; *b*, an apparently healthy plant; *c*, *d*, and *e*, plants in progressive stages of the disease. (Original.)

B.—A spinach field near Norfolk, Va., in which scattered blighted plants occur. As these are yellow, they show light in the illustration. Photographed in January, 1917. (Original.)


A
a
b
c
d
e
B

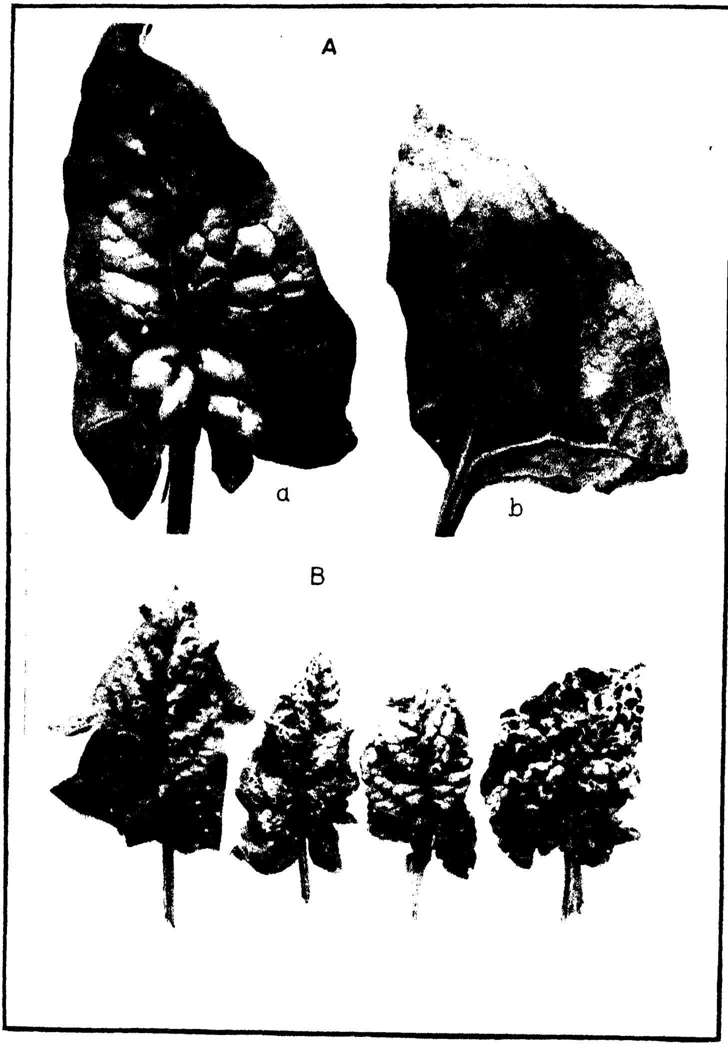

A
a
b
B

PLATE 2

A.—*a*, Upper surface of a spinach leaf affected with downy-mildew (cause: *Peronospora effusa*), showing the light areas which might be mistaken for early symptoms of spinach-blight. *b*, The under surface of a similar leaf. Note the growth of the fungus about the tip. (Original.)

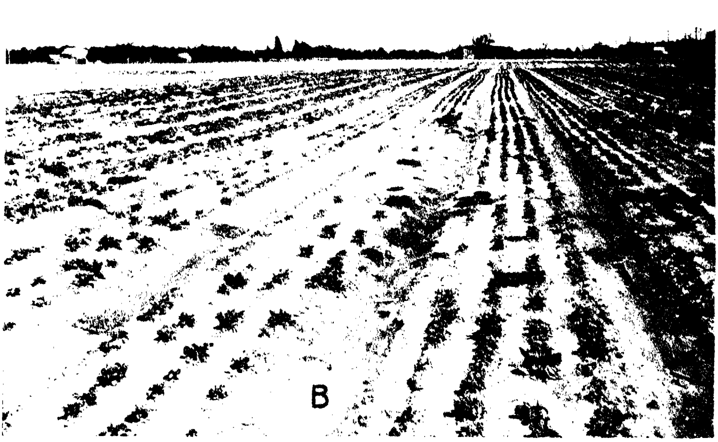
B.—Spinach leaves affected with Heterosporium leafspot. This disease, besides appearing as definite black spots, may cause the leaves to become more or less yellowed, similar in appearance to blighted leaves. (Original.)

PLATE 3

A.—A spinach leaf affected with anthracnose. The degenerating tissues often have the appearance of blighted leaves, but can be distinguished by the presence of fruiting bodies of the causal fungus. Photographed by Mr. Eubanks Carsner.

B.—A spinach field showing blighted spinach plants killed by extreme cold. The healthy plants are alive and green. (Original.)

A



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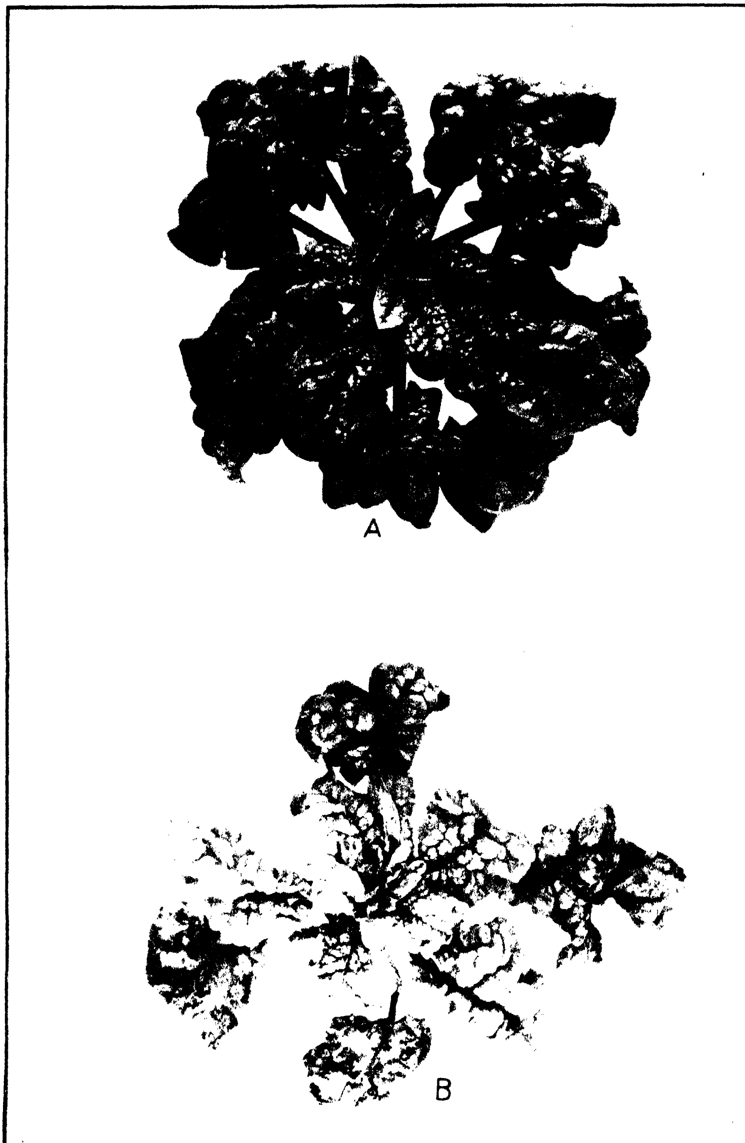
A
B

PLATE 4

A.—A spinach plant showing the first stage of the blight. (Original.)
B.—A spinach plant showing the sixth stage of the blight. (Original.)

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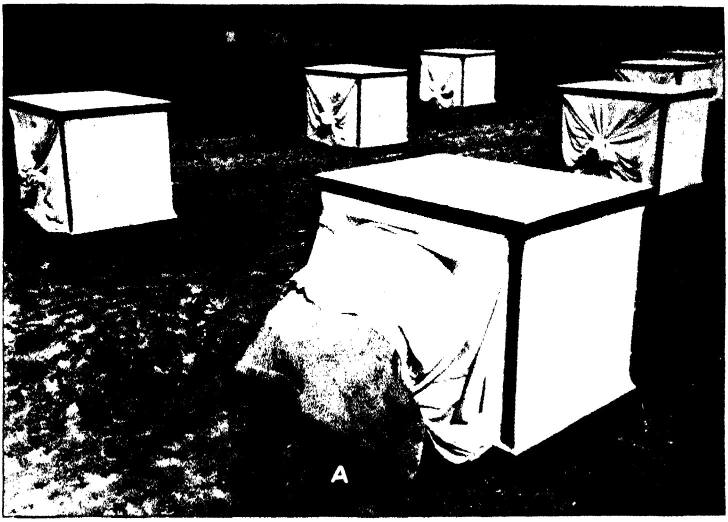
PLATE 5

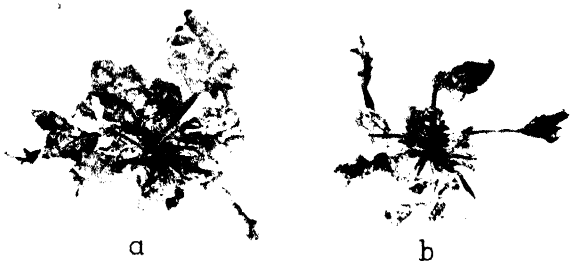
A.—Improved field cage for studying spinach-blight. Note method of draping cloth around body of operator to prevent insects gaining entrance from without. (Original.)

B.—*a*, Seventh stage of spinach-blight; *b*, eighth stage of spinach-blight. (Original.)



A

B



a b


A

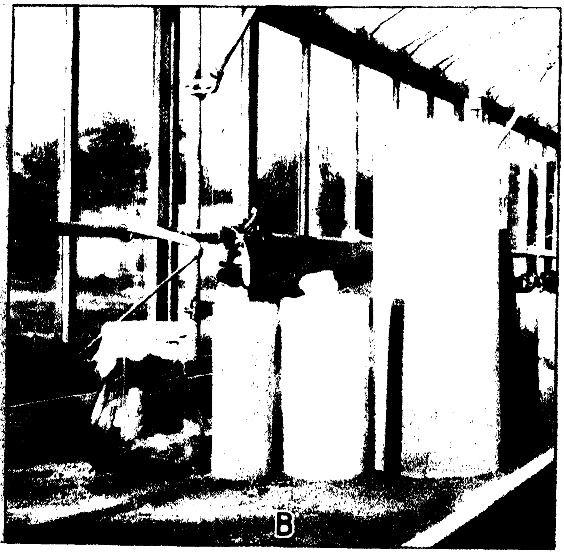

B

PLATE 6

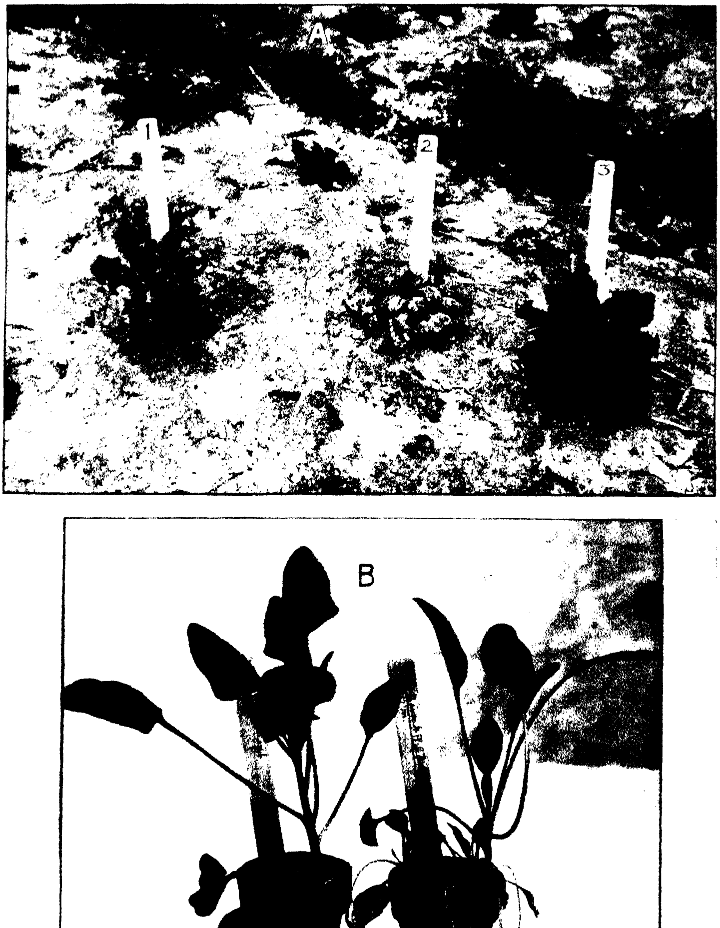
A.—Detail of the door construction of the improved field cage shown in Plate 5, A. The method of overlapping the cloth on one side, thereby avoiding the necessity of sewing the edges, should be noted. The writers are indebted to Mr. J. B. Norton, of the Bureau of Plant Industry, for suggestions relative to the construction of the door. (Original.)

B.—Cloth-covered wire cages, together with a large lantern globe, representative of the types of cage used for greenhouse experiments and for individual inoculations in the field cages. (Original.)

PLATE 7

A.—*1*, Spinach plant showing blotched appearance due to chlorosis. The juice from this plant was not infectious. *2*, Typical fifth-stage blighted plant. The juice from this plant was infectious. *3*, Healthy plant. (Original.)

B.—*a*, Healthy spinach seedling pricked with a flamed needle as a control. *b*, A pot of seedlings inoculated with the virus of spinach-blight 16 days prior to the time this photograph was taken. Note the dwarfed condition of the seedlings and the decidedly mottled leaves. (Original.)


A
1
2
3
B
a
b

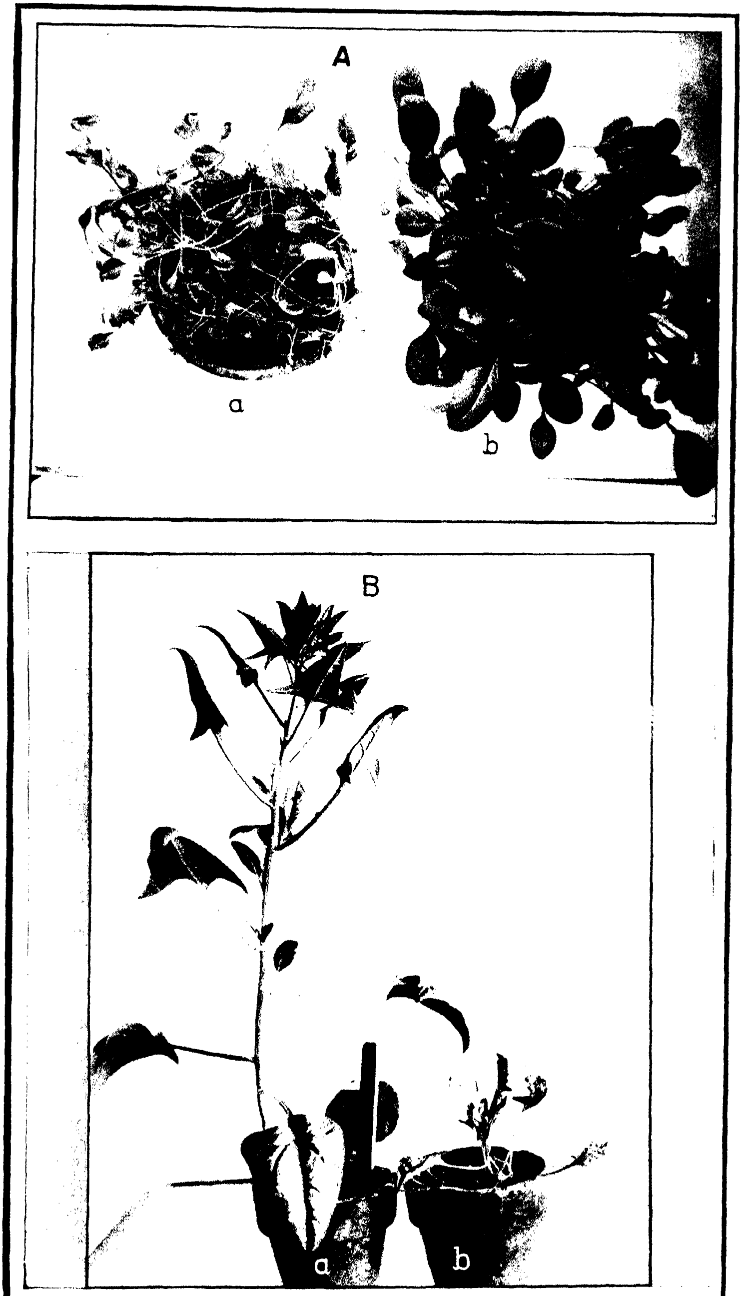

A
a
b
B
a
b

PLATE 8

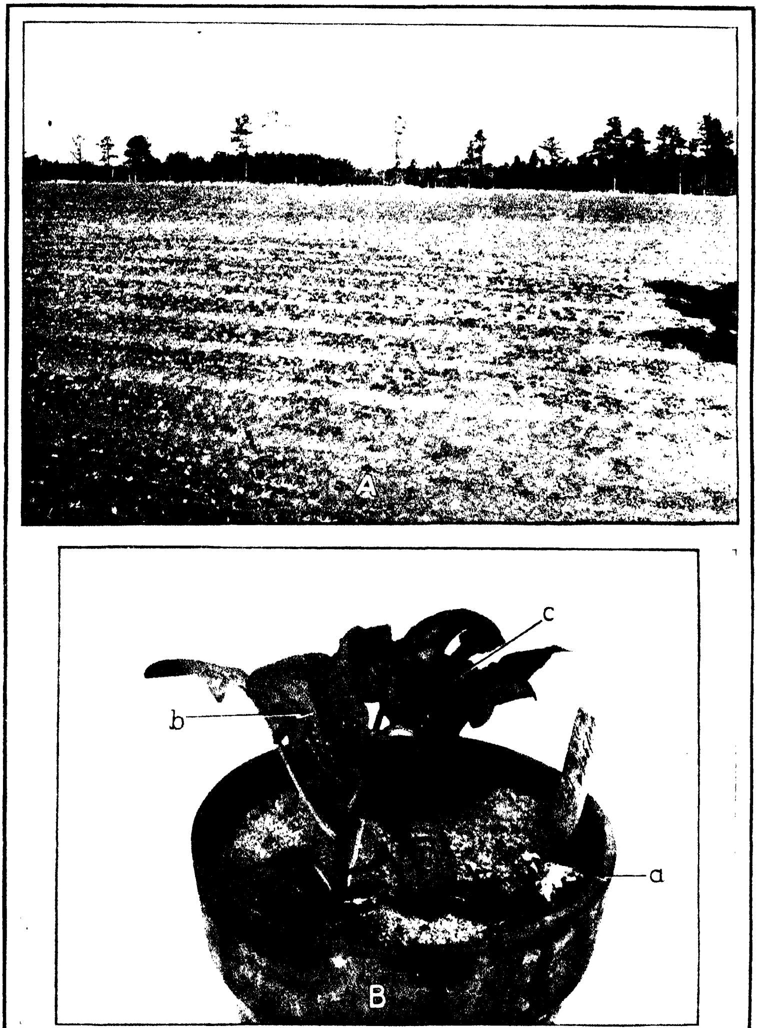
A.—*a*, A pot of spinach seedlings inoculated with virus-bearing aphids collected from blighted plants in the field. All of the plants were infected with blight. Many had died at the time the photograph was taken. *b*, A pot of healthy seedlings which served as controls. (Original.)

B.—*a*, Healthy spinach seedlings used as a control. *b*, Spinach seedlings of the same age as the control, inoculated by needle pricks with the blight virus. (Original.)

PLATE 9

A.—Large area of blighted spinach on farm E, eastern Virginia. (Original.)

B.—Three spinach plants inoculated with virus-bearing *Macrosiphum solanifolii*, first instar. Symptoms on the infected plant (*a*) developed 41 days previous to the time this photograph was taken. The two large plants (*b* and *c*) did not become infected and are healthy. (Original.)


A
c
b
a
B


A
B

PLATE 10

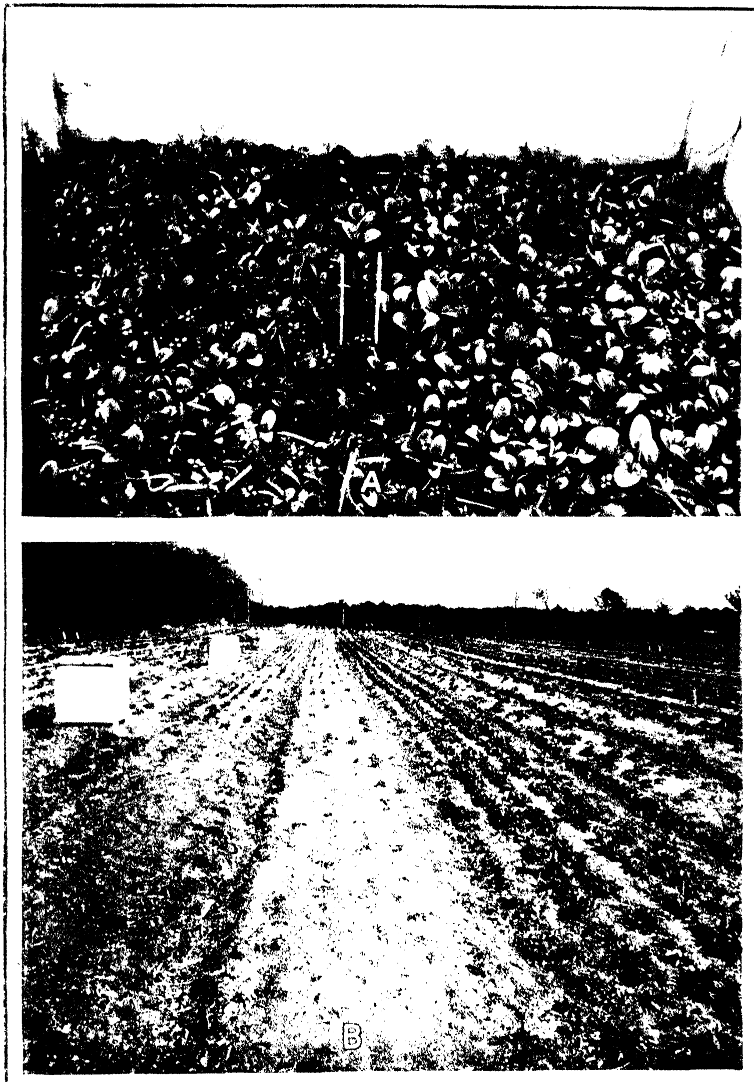
A.—A blighted Viroflay spinach plant from Ohio. (Original.)

B.—Interior of cage 4, in which the aphids and blighted spinach plants were allowed to remain. Note the stunted growth and yellowed cotyledons of the seedlings as compared with those in cage 2 (Pl. 11, A), grown under insect-free conditions. (Original.)

PLATE 11

A.—Interior of cage 2. These spinach plants are the same age as those in cage 4 (Pl. 10, B), but are healthy, as shown by their larger size and deep-green color. (Original.)

B.—Experimental plots. The bed of spinach running from lower right-hand corner of the plate was grown from seed furnished by Mr. J. B. Norton and shows a marked resistance to blight, as compared with the adjoining beds, which were planted with commercial strains of Savoy spinach. (Original.)


A
B

INFLUENCE OF GYPSUM UPON THE SOLUBILITY OF POTASH IN SOILS[1]

By Paul R. McMiller,

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INTRODUCTION

The use of gypsum as a fertilizer was probably familiar to the Romans. Its beneficial effect has been noticed particularly with such field crops as clover and alfalfa, which are especially dependent upon a generous supply of potash, and its action is commonly assumed to be due to an ability to replace potassium in the soil minerals and, hence, to increase the water-soluble portion of this constituent. More recently its favorable effects upon such crops has been attributed by some investigators to its sulphur content. Some of the recently reported laboratory experiments show that applications of gypsum have a very marked effect upon the solubility of potash, while some others indicate that it either has no effect whatsoever or actually decreases the solubility of the potash.

Bradley (*3*)[2] found that gypsum added both to soils from western Oregon and to the mineral pegmatite markedly increased the content of water-soluble potash.

Dumont (*5*), studying the effect of gypsum upon both granitic soils and the separates from these obtained by mechanical analyses, found that when mixed with about one-third its weight of gypsum, moistened, and allowed to stand, the soil gave increasing amounts of water-soluble potash with lengthening periods of contact between soil and gypsum. In the case of the soil separates the fine sand showed an increase of 0.016 part per 1,000 of soil, while the coarser sands and the clay showed no increase even after 34 days' contact.

Morse and Curry (*7, p. 49–50*) found that when powdered feldspar was treated with gypsum the solubility of the potash in water was increased.

Likewise André (*2*) observed a greatly increased solubility of the potash of microcline when this was treated with gypsum.

On the other hand, Fraps (*6*), from an extended laboratory and greenhouse study of the effects of additions of gypsum upon the availability of soil potash, concludes that gypsum is often injurious. He states (*p. 30*):

> Additions of sulphate of lime . . . have no such effect upon rendering potash available to plants as has been claimed. . . .

Most recently of all, Briggs and Breazeale (*4, p. 28*) found that—

> gypsum solutions depressed the solubility of the potassium in orthoclase, the quantity of potash in solution decreasing progressively as the concentration of the calcium sulphate increased.

[1] Published, with the approval of the Director, as Paper 115 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 65–66.

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Using virgin soils from Riverside, California, they found (*p. 28*) that the solubility of the potash was not—

measurably different in distilled water and in solutions of calcium hydrate or calcium sulphate.

Also in a cultivated soil from the same locality they found that the addition of gypsum actually decreased the solubility of the potash.

Apparently in none of the previously reported experiments have the conditions of contact of the gypsum with the soil been similar to those which prevail in the field. In the experiments reported below, the soils, after having the gypsum added, were allowed to remain several months in a condition of moistness similar to that found under field conditions, which, in the case of fine-textured soils in humid regions when evaporation is low and plants are absent, appears to be somewhat below the moisture equivalent (*1, p. 65*).

EXPERIMENTAL WORK

In conducting these experiments the object was to determine whether gypsum, when intimately mixed with soil and kept for some months under conditions of moistness similar to those prevailing in the field, would exert any distinct effect upon the solubility of the potash. For the experiment five soils (Table I), four from different parts of southern Minnesota and one from the Minnesota Experiment Station farm at St. Paul were employed. Sample A from near Wells is a fine-textured soil that would be classified as Fargo clay loam, according to the system of the Bureau of Soils of the United States Department of Agriculture. It is representative of a large area of poorly drained soils of lacustrine origin developed on the late Wisconsin glaciation, being highly calcareous and heavily charged with organic matter. For a soil of this texture it is surprisingly low in total potash.

TABLE I.—*Composition and physical properties of Minnesota soils used in the experiment*

Soil.	Location.	Description of soils.	Reaction.	Moisture equivalent.	Organic matter.[a]	Total Potash.	Calcium carbonate.[b]
					Per cent.	*Per cent.*	*Per cent.*
A.....	Wells...........	Clay loam of lacustrine origin. Surface foot.	Neutral.	38.9	8.48	1.55	4.86
B.....	Spring Valley...	Loam from Kansan till plain. Surface foot.	Acid....	23.8	3.97	1.75	......
C.....	University Farm.	Hempstead silt loam. Surface 6 inches.	Acid....	22.0	48.3	1.78	......
D.....	Worthington....	Silt loam from late Wisconsin till plain. Surface foot.	Neutral.	31.2	5.95	1.93	.75
E.....	Caledonia.......	Knox silt loam. Surface foot.	Acid....	23.5	2.64	2.25	......

[a] Organic matter computed from organic carbon using the formula organic carbon × 1.724=organic matter.
[b] Calcium carbonate computed from carbon dioxid.

Soil B was collected from near Spring Valley and is characteristic of the soils formed on the Kansan drift sheet. It was fairly well supplied with organic matter, but strongly acid in reaction owing to heavy precipitation and age of the drift sheet.

Soil C was taken from the surface 6 inches of the Minnesota Experiment Station farm at St. Paul and is classified as Hempstead silt loam (*8, p. 26*). This soil, overlying beds of sand and gravel, is to be regarded as of alluvial origin deposited from slowly running water issuing from the foot of the retreating ice sheet. It shows an acid reaction and is relatively low in total potash.

Soil D, from near Worthington, would be classified as Barnes silt loam and is representative of a large area of well-drained soil developed on the late Wisconsin drift sheet. It is calcareous, as are all of the soils of this type, and being a prairie soil is relatively high in organic matter.

Soil E is a silt loam from the loess near Caledonia in southeastern Minnesota, and would be classified as Knox silt loam. It is poorly supplied with organic matter, is of a strongly acid character, and is high in total potash.

With the exception of sample C, the soils represent composites of 50 individual samples from the surface foot, 10 taken from each of 5 different virgin fields. Soil C was collected from the surface 6 inches of a small field on University Farm that had been in forest plantation for about 30 years.

PREPARATION OF THE SAMPLES

The air-dried soils were reduced with a rubber pestle so as to pass a 2-mm. sieve. Two 1,000-gm. portions of each were weighed out; 10 gm. of pulverized gypsum were sifted over one, placed on a sheet of oilcloth, and the whole was thoroughly mixed. Enough water was sprinkled over each portion to raise the moisture content to about two-thirds the moisture equivalent, after which they were again thoroughly mixed and finally transferred to glass jars of known weight, and enough water added to raise the moisture content to the moisture equivalent. The jars were kept loosely covered with glass plates to prevent excessive evaporation and allowed to remain in an attic storeroom from February 15 until May 15, 1917. At the end of six weeks the jars were weighed and water added to each until the weight was equal to that at the time they were first put aside. The temperature of the storeroom during this period of exposure varied from 10.5° to 18° C. After this the soils were removed from the jars, spread out upon sheets of oilcloth and allowed to become air-dry when they were passed through a 2-mm. sieve and placed in ordinary Mason jars in which they were kept until the analyses could be begun in the following December.

Four hundred gm. of the air-dried soil were weighed out and placed in a 7-liter bottle and treated with 4,000 c.c. of distilled water. At half-hour

intervals for eight hours the contents of the bottles were thoroughly mixed by vigorous shaking. In the case of the pair of soils from each area the two bottles, the one with the treated and the other with the untreated soil, were placed side by side and shaken at the same time, thus insuring the same degree of agitation and extraction. Then they were allowed to stand for 48 hours, or longer if the most of the clay particles had not settled within that time. Then 3,000 c. c. of the supernatant liquid from each was decanted and filtered. Owing to the presence of colloidal clay in the filtered solutions, especially those from the untreated soils, it was necessary to remove this, which was easily accomplished by bringing the solution to the boiling point, adding 0.5 gm. of aluminium chlorid and 5 c. c. of ammonium-hydroxid solution. The flocculated precipitate of aluminium hydroxid on settling removed from suspension the clay particles. After the solutions had been allowed to stand for several minutes, they were passed through ordinary filter papers, giving clear filtrates. The filtrate thus prepared from each soil and representing a definite quantity of this was then analyzed for potash according to the well-known chloroplatinate method, in which the potassium is weighed as potassium chloroplatinate.

TABLE II.—*Effect of gypsum upon amount of water-soluble potash*

Soil.	Weight of soil corresponding to soil extract used for determination.	Determination.	Untreated soil.		Treated soil.		Increase in potash due to gypsum.
			Weight of potassium chloroplatinate.	Percentage of potash.	Weight of potassium chloroplatinate.	Percentage of potash.	
	Gm.		*Gm.*		*Gm.*		*Per cent.*
A	150	1	0.0262	0.00338	0.0327	0.00422	
A	150	2	.0269	.00347	.0311	.00401	
Average			.0266	.00343	.0319	.00413	0.00070
B	80	1	.0160	.00387	.0260	.00629	
B	80	2	.0149	.00361	.0257	.00621	
Average			.0155	.00374	.0259	.00626	.00252
C	150	1	.0198	.00255	.0309	.00399	
C	150	2	.0196	.00252	.0297	.00383	
Average			.0197	.00254	.0303	.00391	.00137
D	80	1	.0098	.00237	.0208	.00503	
D	80	2	.0112	.00271	.0178	.00431	
Average			.0105	.00254	.0193	.00467	.00213
E	80	1	.0180	.00436	.0362	.00876	
E	80	2	.0200	.00489	.0336	.00813	
Average			.0190	.00462	.0349	.00845	.00383

In Table II are reported the amounts of soil equivalent to the solution used for each determination, the weight of the potassium chloroplatinate (K_2PtCl_6), and the percentage of the potassium computed as potash (K_2O). Duplicate determinations are reported to show the degree of concordance. The amount of chloroplatinate in each determination was so large as to eliminate the large experimental errors of weighing that occur when only small amounts of soil are employed, while the duplicates in all cases are closely concordant. A blank determination was made with the same kind and quantities of reagents as were used in the actual analyses and the proper corrections made; the gypsum was analyzed and was found to be quite free of potash.

In the case of each soil there is shown a marked increase, due to the addition of the gypsum. The greatest increase was found in the case of soil E in which the gain amounted to over 80 per cent and the least in the case of soil A 20 per cent. While the amount of gypsum employed in the experiment, 1 per cent, equivalent to 10 tons per acre, was much larger than is used in field practice, it would be surprising, in view of the results obtained, if a light application did not cause an appreciable increase in the water-soluble potash.

SUMMARY

Various Minnesota soils when mixed with 1 per cent of gypsum, raised to a point approximating the moisture equivalent, and kept in this condition for three months showed marked increases in the content of water-soluble potash.

The results in previously reported experiments by various investigators in which the action of gypsum has not been found to cause such an increase may be due to the conditions of contact between the soil and gypsum that they have employed being unlike those that obtain in the field.

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CORRELATION BETWEEN THE PERCENTAGE OF FAT IN COW'S MILK AND THE YIELD[1]

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INTRODUCTION

It is a generally accepted opinion that cows with a large yield of milk produce a smaller percentage of fat than do cows with a small yield of milk. Stated in another way, it is thought that low-yielding cows produce a higher percentage of fat than that produced by high-yielding cows.

To what extent this is true or not true has not up to the present time been demonstrated by a careful statistical investigation. Wilson [2] from a study of the records of 2,866 Ayrshire cows concluded that quantity and quality (yield of milk and percentage of fat) were independent of each other. He states:

> If we group together all the low-yielding cows, and find their milk invariably high in quality, we may infer that low yield and high quality are of the nature of concomitant variations. If we group the high-yielding cows together, and find their milk invariably of low quality, we may infer that high yield and low quality run together. But if we take these groups and any other groups we can form, and find that the quality varies the same way in them all—that is that there are low qualities, high qualities, and medium qualities in every one of them—then we are justified in inferring that the quantity and quality of the milk are independent of each other. And this is what we do find.

In a criticism of this work Pearson,[3] by means of a correlation table, showed that there was a small but significant decrease in the percentage of fat with an increase in the yield of milk, and pointed out the fallacy of such a process of reasoning in connection with statistical data.

[1] Paper No. 5 from the Laboratory of Genetics, Agricultural Experiment Station of the University of Illinois.

[2] WILSON, James. THE SEPARATE INHERITANCE OF QUANTITY AND QUALITY IN COWS' MILK. *In* Sci. Proc. Roy. Dublin Soc., n. s., v. 12, no. 33, p. 470–479, 6 diagr. 1910.

[3] PEARSON, Karl. NOTE ON THE SEPARATE INHERITANCE OF QUANTITY AND QUALITY IN COWS' MILK. *In* Biometrika, v. 7, No. 4, p. 548–550. 1910.

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Wilson did not handle his data in such a way as to bring out the relationship which exists between the quantity and quality. In "The Principles of Stock-Breeding," Wilson [1] again writes:

> In connection with yield and quality in milk, it has been assumed, frequently, that the two characters are interdependent: that when the one is high the other must be low. It has been found that this is not so. The characters are independent and have no effective influence upon each other. High quality of milk is found among cows giving all kinds of yield, and low quality is found similarly.

It seemed to the writer that it might be of some value to make a more careful statistical investigation of this question with our American cattle.

SOURCE OF DATA

In the registers of the different American associations is to be found a large body of data which furnished the major part of the material for this investigation. There are involved in this study the following: 2,141 yearly tests of Jerseys, Register of Merit, 1911, 1913; 3,564 Guernseys, Guernsey Breeders' Journal, May, 1915; 1,925 Holstein-Friesians, Holstein-Friesian Advanced Register Year Book, volumes 21–26; 1,091 Ayrshires, Year Book of the Ayrshire Breeders' Association, 1907, 1911, 1913, 1914; 98 Ayrshires [2]; 750 grade Jerseys [3] and 341 grade Holstein-Friesians [4]; and 2,002 [4] yearly tests of cows unclassified as to breed.

Only the yearly tests were used for the reason that a yearly record is a more reliable criterion of a cow's performance and ability than a shorter test. It should be pointed out here, that in the case of the records from the associations, selected groups of individuals are involved in this study, since only selected individuals are subject to entry in the registers of the associations.

The method of finding the relation between the percentage of fat and the yield of milk is by means of the correlation table. The cows are grouped, according to age when the test began, into the following groups: 2 to 3 years, 3 to 4 years, 4 to 5 years, and 5 years and over. The last group comprises what are usually held to be mature cows. These are not exact divisions according to age, since a given group may contain individuals differing in age by almost a year. For example, the 3-to-4-year group contains those cows with tests beginning at some time after they were 3 and before they were 4 years old. A cow with a test beginning the day she was 3 would be practically a year younger than one having a test starting when she was one day under 4, though both would be classed in the same group. Of course, there are few cases of this kind.

[1] WILSON, James. THE PRINCIPLES OF STOCK-BREEDING. p. 121–122. London, 1912.
[2] Furnished by Mr. C. M. Winslow, Secretary of the Ayrshire Breeders' Association.
[3] Obtained from Mr. W. W. Yapp, Illinois Agricultural Experiment Station.
[4] Obtained from Prof. W. J. Fraser, Illinois Agricultural Experiment Station.

POSSIBLE SOURCES OF ERROR

In some of the breeds, Jersey and Guernsey, a yearly test consists of any 365 consecutive days. This may cover parts of two lactation periods, which is not a serious objection, since the study is interested in the relation between the percentage of butter fat and yield of milk in groups of individuals and not in the individual herself. In other words, the question is to what extent, if any, do cows with large milk yield tend to show a low percentage of fat, or cows with a low milk yield to show a high percentage. The Ayrshire Association specifies both the amount of milk and butter fat necessary for entrance. In the other associations only the butter-fat yield is specified. Unless the requirements of the Ayrshire Association are in accordance with the natural relation of butter-fat and milk yield, one would expect to find abnormal results in such a selected group, which would not hold for Ayrshire cattle in general. This point will be treated more fully later in this paper. For the grade Jerseys, grade Holstein-Friesians, and cows unclassified as to breed, it should be pointed out that the populations are composed of a heterogeneous lot, and whatever results are found will apply only to such mixed populations.

ANALYSIS OF DATA

JERSEY

Tables I to IV show, in the form of correlation tables, the distribution of individuals with regard to the yield of milk and the percentage of fat. Table V contains all the tests of Jerseys regardless of age and was made by combining Tables I to IV. Text Table A summarizes the means, standard deviations, coefficients of variability of milk and fat, and the correlation between the percentage of fat and the yield for Jerseys of different ages, and for Jerseys, irrespective of age.

TABLE A.—*Summary of results from a study of the correlation between the percentage of fat and yield of milk for Jerseys*

[Fat in percentage; milk in pounds]

Age.	Number of animals.	Type of test.	Mean.	Standard deviation.	Coefficient of variability.	Correlation.
Years.						
2 to 3	877	Milk	6,475.0 ±28.9	1,270.5 ±20.5	19.62±0.33	−0.360±0.020
		Fat	5.425± 0.012	0.517± 0.008	9.5[illegible]±0.15	
3 to 4	411	Milk	7,325.0 ±45.8	1,377.5 ±32.4	18.81±0.46	−0.437±0.027
		Fat	5.401± 0.019	0.562± 0.013	10.41±0.25	
4 to 5	219	Milk	8,043.4 ±60.7	1,332.5 ±42.9	16.57±0.55	−0.359±0.040
		Fat	5.462± 0.024	0.533± 0.017	9.76±0.32	
5 and over	634	Milk	8,814.5 ±43.1	1,608.5 ±30.5	18.25±0.35	−0.397±0.023
		Fat	5.322± 0.013	0.502± 0.010	9.44±0.18	
All ages	2,141	Milk	7,491.4 ±25.0	1,718.0 ±17.7	22.93±0.25	−0.354±0.013
		Fat	5.392± 0.008	0.525± 0.005	9.74±0.10	

The correlation is negative and very significant for all ages. When judged by their probable errors, there are no significant differences among the correlations for the different groups.

The milk yield increases from an average of 6,475 pounds for the 2-to-3-year-old class to 8,814.5 pounds for the mature class (fig. 1). Since there is a marked negative correlation between the percentage of fat and yield at all ages, one might look for the percentage of fat to decrease as the yield of milk increases with the age of the cows. The class which is 5 years and over shows a slightly smaller percentage of fat, but the 4-to-5-year-old class has a higher percentage than the 3-to-4-year-old class, though the difference is not significant (fig. 2). On the whole, the percentage remains practically the same. This may be due to the relatively stable relation between the amount of fat and yield throughout the growing period of the individual cow. Holdaway[1] found this to be true for Holstein-Friesians, using the 7-day records. Stated in another way, the percentage of fat seems to be fairly constant throughout the life of a given individual, but different individuals show differing percentages of fat. The results show that at a given age cows with a high milk yield tend to produce a lower percentage of fat than do cows with a low milk yield. This is expressed by the negative correlations obtained. Table B illustrates in another way that this is true. This table is the result of arbitrarily dividing Table II into three parts, cows yielding 4,500 to 6,500, those yielding 7,000 to 9,000,

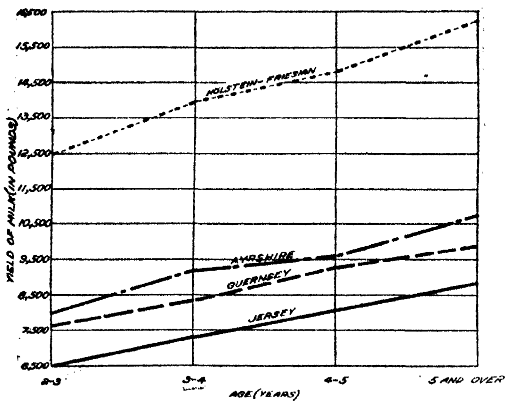

FIG. 1.—Graphs showing the averages of the milk yield for the different ages of cows.

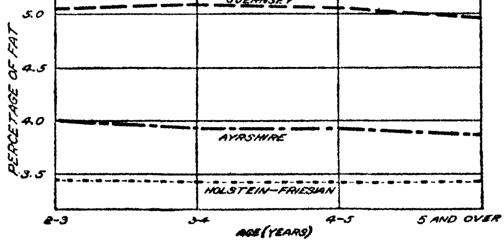

FIG. 2.—Graphs showing the averages of percentages of butter fat for different breeds of cows.

[1] HOLDAWAY, C. W. STATISTICAL WEIGHTING FOR AGE OF ADVANCED REGISTRY COWS. *In* Amer. Nat., v. 50, no. 599, p. 676–687, 2 fig. 1916.

and those yielding 9,500 to 13,000 pounds of milk, and finding the average yield of milk and percentage of fat. The decrease from 5.657 per cent for the first group to 4.941 per cent for the third group is very significant. Any group of cows showing a negative correlation between percentage of fat and yield of milk would give, in general, the same results if treated as was Table II.

TABLE B.—*Milk yield and percentage of butter fat of Jerseys 3 to 4 years of age*

Number of animals.	Extremes of milk yield.	Average milk yield.	Average percentage of fat.
	Pounds.	*Pounds.*	
152	4,500– 6,500	6,049.3	5.657
220	7,000– 9,000	7,677.3	5.307
39	9,500–13,000	10,307.7	4.941

GUERNSEY

Tables VI to IX show the distribution of individuals with regard to the yield of milk and the percentage of butter fat, in the form of correlation tables. Table X combines Tables VI to IX. Table C gives the means, standard deviations, coefficients of variability of milk and fat, and the correlation between the percentage of fat and yield of milk for Guernseys of different ages.

TABLE C.—*Summary of results from a study of the correlation between the percentage of fat and yield of milk for Guernseys*

[Fat in percentage; milk in pounds]

Age.	Number of animals.	Type of test.	Mean.	Standard deviation.	Coefficient of variability.	Correlation.
Years.						
2 to 3	1,375	Milk	7,608.0 ±28.8	1,584.0 ±20.4	20.82±0.77	−0.251±0.017
		Fat	5.065± 0.008	0.458± 0.006	9.03±0.32	
3 to 4	644	Milk	8,317.0 ±49.3	1,854.0 ±34.8	22.29±0.44	−0.289±0.024
		Fat	5.080± 0.013	0.477± 0.009	9.39±0.18	
4 to 5	478	Milk	9,247.0 ±62.0	2,030.0 ±44.3	21.95±0.50	−0.264±0.029
		Fat	5.046± 0.014	0.462± 0.010	9.13±0.20	
5 and over	1,067	Milk	9,893.0 ±42.8	2,067.5 ±30.2	20.90±0.68	−0.337±0.018
		Fat	4.956± 0.010	0.478± 0.007	9.64±0.30	
All ages	3,564	Milk	8,644.4 ±23.7	2,095.4 ±16.7	24.24±0.20	−0.296±0.010
		Fat	5.033± 0.005	0.471± 0.004	9.35±0.08	

The correlation coefficients are negative as in the case of the Jerseys, but slightly smaller. For the Jerseys they range from -0.359 ± 0.040 to -0.437 ± 0.027, while for the Guernseys the range is from -0.251 ± 0.017 to -0.337 ± 0.018. The yield of milk gradually increases from an average of 7,608 pounds for the 2-to-3-year-old class to 9,893 for the group which is 5 years and over. The average percentage of fat varies from 4.956 ± 0.010 for the group which is 5 years and over to 5.080 ± 0.013 for the 3-to-4-year-old class. (See fig. 2.)

HOLSTEIN-FRIESIAN

Tables XI to XIV exhibit the distributions of individuals with regard to the yield of milk and the percentage of fat in the form of correlation tables. Table XV combines Tables XI to XIV. Table D summarizes the means, standard deviations, coefficients of variability for milk and fat, and the correlations between percentage of fat and yield for Holstein-Friesians of different ages.

TABLE D.—*Summary of results from a study of the correlation between the percentage of fat and yield of milk for Holstein-Friesians*

[Fat in percentage; milk in pounds]

Age.	Number of animals.	Type of test.	Mean	Standard deviation.	Coefficient of variability.	Correlation.
Years.						
2 to 3	610	Milk	12,488.5 ± 80.4	2,942.0 ± 56.8	23.56 ± 0.48	−0.116 ± 0.027
		Fat	3.462 ± 0.008	0.294 ± 0.006	8.50 ± 0.16	
3 to 4	341	Milk	13,938.5 ± 116.5	3,188.0 ± 82.4	22.87 ± 0.62	−0.160 ± 0.039
		Fat	3.433 ± 0.012	0.322 ± 0.008	9.38 ± 0.24	
4 to 5	292	Milk	14,825.5 ± 124.4	3,151.0 ± 87.9	21.25 ± 0.62	−0.088 ± 0.039
		Fat	3.417 ± 0.012	0.313 ± 0.009	9.17 ± 0.26	
5 and over	682	Milk	16,280.0 ± 94.4	3,654.5 ± 66.7	22.45 ± 0.43	−0.115 ± 0.026
		Fat	3.420 ± 0.008	0.301 ± 0.006	8.81 ± 0.16	
All ages	1,925	Milk	14,443.1 ± 56.0	3,640.7 ± 39.6	25.21 ± 0.29	−0.133 ± 0.015
		Fat	3.435 ± 0.005	0.305 ± 0.003	8.88 ± 0.10	

The correlations are much smaller for the Holstein-Friesian than for the two preceding breeds. They range from -0.088 ± 0.039 to -0.160 ± 0.036. The first is not significant when judged by the probable error. The milk yields increase regularly from 12,488 pounds for the youngest class to 16,280 for the oldest. The percentages of fat remain practically constant for the different ages. (See fig. 2.)

AYRSHIRE

Tables XVI to XIX are correlation tables for the different classes of Ayrshires. Table E gives means, standards deviations, coefficients of variability for milk and fat, and the correlations between percentages of fat and yield of milk.

TABLE E.—*Summary of results from a study of the correlation between the percentage of fat and yield of milk for Ayrshires*

[Fat in percentage; milk in pounds]

Age.	Number of animals.	Type of test.	Mean.	Standard deviation.	Coefficient of variability.	Correlation.
Years.						
2 to 3	343	Milk	7,960.5 ± 47.9	1,314.5 ± 33.9	16.51 ± 0.44	+0.019 ± 0.036
		Fat	4.016 ± 0.011	0.310 ± 0.008	7.71 ± 0.20	
3 to 4	192	Milk	9,179.5 ± 86.1	1,769.5 ± 60.9	19.28 ± 0.69	−0.093 ± 0.048
		Fat	3.936 ± 0.014	0.314 ± 0.011	6.35 ± 0.28	
4 to 5	160	Milk	9,584.5 ± 83.5	1,566.5 ± 59.1	16.34 ± 0.63	−0.023 ± 0.053
		Fat	3.919 ± 0.016	0.303 ± 0.011	7.73 ± 0.29	
5 and over	396	Milk	10,726.0 ± 67.1	1,980.0 ± 47.5	18.46 ± 0.46	−0.047 ± 0.034
		Fat	3.865 ± 0.011	0.313 ± 0.008	8.10 ± 0.19	
All ages	1,091	Milk	9,417.1 ± 41.7	2,044.4 ± 29.5	21.71 ± 0.33	−0.138 ± 0.020
		Fat	3.933 ± 0.006	0.318 ± 0.005	8.08 ± 0.12	

In no one of the four groups classified as to age is there a significant correlation, meaning that for these classes of individuals studied the milk yield and percentage of fat are independent. It is curious that this breed should have a different relation existing between fat and milk than that of the Jerseys, Guernseys, and Holstein-Friesians. This result is probably due to the fact that they are a much more highly selected group of individuals than in the case of any of the other breeds, owing to the requirements for registry imposed by the Ayrshire Association. The minimum requirements are as follows:

Age.	Weight of milk.	Weight of fat.
Years.	*Pounds.*	*Pounds.*
2 to 3	6, 000	214. 3
3 to 4	6, 500	236. 0
4 to 5	7, 500	279. 0
5 and over	8, 500	322. 0

This would mean that upon the basis of these specified amounts the percentages of fat for the different ages would be as follows:

2 to 3 years	3. 572
3 to 4 years	3. 631
4 to 5 years	3. 720
5 years and over	3. 788

A gradual increase in the percentage of fat as the age increases is to be noted. This is contrary to what was found to exist for the other breeds. For Table XX, constructed from Tables XVI to XIX, a correlation of -0.138 ± 0.020 is found. This is practically the same correlation found for the Holstein-Friesian cows. This negative correlation resulting from combining the subgroups of Ayrshires is to be expected, since there is an increase in the milk yield with age and a decrease in the percentage of fat. (See Table E.) It may be that the requirements for entry in the register of the Ayrshire Association tend to eliminate high-yielding cows with a low percentage of fat and low-yielding cows with a high percentage of fat.

The correlation between the yield and percentage of fat for 98 Ayrshire cows which were tested and failed to meet the requirements was found to be

$$r = -0.226 \pm 0.065.$$

A larger number of such tests would be necessary to determine whether a difference exists between cows which meet the requirements and those which do not.

The percentage of butter fat among these 98 tests decreases with age as in Table E, as follows:

Number of animals.	Age.	Percentage of fat.
	Years.	
19	2 to 3	4.112
22	3 to 4	4.035
20	4 to 5	3.997
37	5 and over	3.842

The work of Speir [1] shows that there is a slight tendency for the percentage of fat to decrease in Ayrshires after the third year. Both Jerseys and Guernseys of 5 years of age and over show a slight decrease. (See Tables A and B.) The mature class of Holstein-Friesian cows, however, does not show a decrease (Table C).

COWS NOT PURE-BRED

The writer thought it would be of interest to see the extent of correlation between the yield and percentage of fat in cows not pure-bred. Grade Jerseys, grade Holsteins-Friesians, and cows unclassified as to breed are considered.

Tables F, G, and H give the results of the study for the different classes in the order given above.

TABLE F.—*Summary of results from a study of the correlation between the percentage of fat and yield of milk for grade Jerseys*

[Fat in percentage; milk in pounds]

Age.	Number of animals.	Type of test.	Mean.	Standard deviation.	Coefficient of variability.	Correlation.
Years.						
2 to 3	101	Milk	4,663.5 ±81.7	1,217.0 ±57.8	26.10±1.32	−0.114±0.066
		Fat	5.106± 0.033	0.489± 0.023	9.57±0.45	
3 to 4	155	Milk	5,035.5 ±71.3	1,316.0 ±50.4	26.13±1.07	−0.234±0.051
		Fat	5.124± 0.023	0.592± 0.023	11.56±0.45	
4 to 5	146	Milk	5,298.0 ±71.5	1,281.5 ±50.6	24.19±1.01	−0.272±0.052
		Fat	5.068± 0.029	0.520± 0.021	10.26±0.41	
5 and over	348	Milk	5,504.5 ±48.1	1,329.0 ±34.0	24.14±0.65	−0.138±0.035
		Fat	4.923± 0.022	0.606± 0.016	12.32±0.32	

[1] SPEIR, John. MILK RECORDS. *In* Trans. Highland and Agr. Soc. Scotland, s. 5, v. 16, p. 170–229, fig. 18–20. 1904.

TABLE G.—*Summary of results from a study of the correlation between the percentage of fat and yield of milk for grade Holstein-Friesians*

[Fat in percentage; milk in pounds]

Age.	Number of animals.	Type of test.	Mean.	Standard deviation.	Coefficient of variability.	Correlation.
Years.						
2 to 3	76	Milk	5,776.5 ± 113.4	1,465.5 ± 80.2	25.37±1.48	−0.211±0.074
		Fat	3.688± 0.028	0.361± 0.020	9.80±0.54	
3 to 4	88	Milk	6,727.5 ± 112.1	1,559.0 ± 79.3	23.17±1.24	−0.345±0.063
		Fat	3.568± 0.025	0.344± 0.018	9.64±0.49	
4 to 5	41	Milk	7,305.0 ± 214.8	2,039.5 ± 151.9	27.92±2.23	−0.155±0.103
		Fat	3.581± 0.041	0.391± 0.029	10.92±0.82	
5 and over	136	Milk	7,441.0 ± 104.1	1,799.0 ± 73.6	24.18±1.05	−0.212±0.058
		Fat	3.546± 0.025	0.440± 0.018	12.39±0.51	

TABLE H.—*Summary of results from a study of the correlation between the percentage of fat and yield of milk for cows unclassified as to breed*

[Fat in percentage; milk in pounds]

Age.	Number of animals.	Type of test.	Mean.	Standard deviation.	Coefficient of variability.	Correlation.
All ages	2,002	Milk	5,824.6 ± 28.5	1,888.2 ± 20.1	32.42±0.38	−0.359±0.013
		Fat	3.902± 0.009	0.575± 0.006	14.75±0.16	

There is a very significant negative correlation between the yield and percentage of fat for all three classes of cows represented by the three foregoing tables (Tables F–H).

CONCLUSIONS

(1) A significant negative correlation exists between the percentage of fat in cows' milk and the yield for the Jerseys, Guernseys, Holstein-Friesians, grade Jerseys, grade Holstein-Friesians, and cows unclassified as to breed. The correlation for Ayrshires is not significant in the subgroups classed in respect to age, but it is significant when these groups are treated as a whole. (See Tables XXI and F, G, H.)

(2) The yield of milk increases with age. However, since all cows 5 years of age and over are classed together, it may well be that the yield decreases at some period beyond 5 years. Pearl and Patterson [1] showed that in Jersey cows using the 7-day records that the maximum production is reached between the eighth and ninth year. Crowther [2] from records of Ayrshires is of the opinion that maximum production is close to the eighth year. (See Table XXI and fig. 1.)

(3) In the Jerseys, Guernseys, and Holstein-Friesians the percentage of fat remains fairly constant for the different ages studied. However,

[1] PEARL, Raymond, and PATTERSON, S. W. THE CHANGE OF MILK FLOW WITH AGE, AS DETERMINED FROM THE SEVEN-DAY RECORDS OF JERSEY COWS. Maine Agr. Exp. Sta. Bul. 262, p. 145–152, fig. 7. 1917.

[2] CROWTHER, Charles. VARIATION IN THE COMPOSITION OF COW'S MILK. *In* Jour. Agr. Sci., v. 1, pt. 2, p. 149–175. 1905.

the group 5 years of age and over in the Jerseys and Guernseys shows a slightly lower percentage of fat than the younger groups. In the case of the Ayrshires, there is a gradual decrease with age. Between the youngest and oldest groups there is a difference of 0.151 per cent. (See Table XXI and fig. 2.)

(4) When judged by the standard deviation, age has no influence on the variability of the percentage of butter fat. But the class 5 years of age and over is more variable in the yield of milk than the younger groups. This may occur because of the inclusion in this group of old cows whose milk yield has decreased. (See Table XXI.)

(5) The breed has an influence on the variability of milk yield and percentage of fat, using the standard deviation as a basis of comparison. For variability in yield the breeds stand in the following order in an ascending scale: Jersey, Ayrshire, and Guernsey practically the same, Holstein-Friesian. For percentage of fat, the order is: Holstein-Friesian and Ayrshire about the same, Guernsey, Jersey. (See Table XXI.)

(6) For the production of milk the breeds stand as follows (see Table XXI.)

	Pounds.
Holstein-Friesian	14,443.1
Ayrshire	9,417.1
Guernsey	8,644.4
Jersey	7,491.4

(7) The average percentages of fat for the different breeds are as follows (see Table XXI.)

	Pounds.
Jersey	5.392
Guernsey	5.033
Ayrshire	3.933
Holstein-Friesian	3.435

TABLE I.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Jerseys 2 to 3 years of age*

Yield of milk, in pounds.	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.0	Total.
4,000																						2	1		2	1	3		1	10
4,500															1	2	2	2	5	7	4	3	2		2					30
5,000								2	1	1	11	3	12	6	11	8	4	9	9	6	5	1	5	2		1			1	98
5,500				1	3	2	6	8	4	7	7	12	15	10	10	7	7	12	5	2	3		4	4	1					130
6,000			1	1	2	3	11	8	10	13	11	13	10	16	8	8	15	7	5	2	2	6			2		1			155
6,500			5	2	5	6	5	7	8	14	7	11	10	9	7	7	10	6	3	4	3		2	1						132
7,000	1	1	2	4	5	4	5	6	7	7	13	10	5	14	4	3	6	4	1	5	3		2							112
7,500	1	1	3	3	2	5	3	5	4	4	9	10	7	3	3	6	5	6	2	3	1	1								87
8,000		1	1	4	1	4	6		5	2	2	5	3	3	2	3	6	1		1		1								51
8,500		1	2	1		1	2	1	5	5	4	1	2	3			1	1												30
9,000			1	1		1	2			3	2	1	1						1	1										14
9,500				1					2	1	3	1	1			1	2		1											13
10,000				1	1				1				1		1						1									6
10,500							1	1		2																				4
11,000					1								1				1													3
11,500																														0
12,000																														0
12,500																									1					1
13,000																														0
13,500																														0
14,000			1																											1
Total...	2	4	16	19	20	20	41	38	47	59	69	67	68	64	47	45	59	48	32	31	22	14	16	7	8	2	4	0	2	877

Percentage of fat (column heads)

$r = -0.360 \pm 0.020$

TABLE II.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Jerseys 3 to 4 years of age*

Percentage of fat

Yield of milk, in pounds	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	Total.
4,500	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	11
5,000	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	1	3	1	...	...	1	...	2	...	...	...	...	...	...	1	...	...	20
5,500	...	...	...	...	...	...	...	...	...	...	...	...	...	2	2	2	2	2	4	3	3	2	1	...	1	...	1	...	2	...	...	...	...	...	47
6,000	...	...	...	...	...	...	...	1	...	...	1	2	2	12	2	5	5	3	2	2	1	3	2	1	1	1	...	1	2	...	...	...	...	...	69
6,500	...	...	...	...	...	...	...	1	1	2	5	7	6	7	5	3	7	3	4	5	2	1	3	1	1	...	...	...	...	...	...	1	...	...	75
7,000	...	...	...	...	...	3	5	1	3	6	2	3	6	4	5	6	3	2	4	4	5	5	1	2	...	...	...	...	1	...	...	...	...	...	71
7,500	1	1	...	...	1	1	2	2	3	2	5	3	11	2	2	8	2	6	4	4	3	1	...	1	1	...	...	...	1	...	...	...	...	...	67
8,000	...	...	1	...	2	1	...	3	1	5	3	1	3	1	4	1	4	1	...	1	1	2	...	...	...	...	...	...	...	...	...	...	...	...	35
8,500	...	...	1	1	1	...	3	...	1	2	4	...	2	1	2	3	...	...	1	1	3	...	1	...	...	...	...	...	...	...	...	...	...	...	27
9,000	...	...	...	1	...	1	2	...	1	2	1	4	3	...	...	1	1	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	20
9,500	...	...	1	1	...	...	2	1	1	...	2	1	1	2	1	...	...	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	15
10,000	...	...	...	1	...	2	...	...	1	...	1	...	...	...	1	1	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	8
10,500	...	...	...	...	...	1	...	...	2	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	5
11,000	...	...	...	...	...	...	...	1	1	1	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4
11,500	...	...	...	...	...	...	...	2	1	...	...	...	...	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	5
12,000	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
12,500	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
13,000	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
Total	1	1	3	4	5	9	14	12	17	21	24	22	34	33	24	32	25	21	22	22	18	14	10	5	6	1	1	1	6	0	0	2	0	1	411

$r = -0.437 \pm 0.027$

TABLE III.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Jerseys 4 to 5 years of age*

Yield of milk, in pounds	Percentage of fat																									
	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	Total.
5,500																	1						1			2
6,000													4	2	2				1	1	2		1	1	1	15
6,500								1	1	2	3	1	2		1	2	1	1	1		1	1				18
7,000					1		1	1	4	1	1	4	5	2	1	2			2	3	1		1			30
7,500		1		1	2	1	6	2	3	2	2	2		2	1	1	1		5				1			33
8,000		2		1		4	4	1	3	4	1	4			3	3	3	2	2							37
8,500	2	1			2	4	2		2	2	5	2	1	3					1							27
9,000			2		1			1		2		1		1	1	1	1				1		1			13
9,500			2	1	1	3	2		3	2					1	1			1							17
10,000		1	2				4	2		1		1		2	2		1									16
10,500							2	1				1	1				1									6
11,000	1												1													2
11,500					1						1															2
12,000																										0
12,500																										0
13,000																										0
13,500																										0
14,000																1										1
Total	3	5	6	3	8	12	21	9	16	16	13	16	14	12	12	11	9	3	13	4	5	1	5	1	1	219

$r = -0.359 \pm 0.040$.

TABLE IV.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Jerseys 5 years and over*

Percentage of fat

Yield of milk, in pounds	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	Total.
5,500																										2			1	3
6,000																					2	1			2	1	2			8
6,500															1	4	1	3	3	1	2		2	1			1			19
7,000											1	6	6	10	14	4	3	2	5	3	4	4	2		1			1		66
7,500								1	1	2	3	5	6	4	5	6	8	7	4	3	6	2		1		1	1			66
8,000					1		2	3	3	5	5	11	10	8	13	7	8	5	5	5	5	1	4		1			1		103
8,500				1		2	2	4	3	9	9	9	7	5	3	9	10	3	3	2	2			1	1					85
9,000			1	1	3	3	4	3	5	5	8	8	5	6	7	5	3	1	1	3	2	1	1							76
9,500				2		3	6	4	3	4	7	1	1	4	8	4	2	3	2	3		1	1	1						60
10,000					1	1	1		5	5	3	3		5	3	4	1	1	4	1		1	1	1					1	42
10,500	1	1	1		2			1	4	3	3	2	3	4	3		2	1	2		2	2								37
11,000			2	2	1	1	2	1	1		3		2	1	1	2						1								20
11,500				1	1	2		2	2	1	2	1		1					1	1	1									16
12,000						1	2			1		1	1	1						1			1							9
12,500								1	1	1	2	2			1															8
13,000										1				1		1			1			1								5
13,500						1		1																						2
14,000						1			1	1	2																			5
14,500						1		1																						2
15,000										1																				1
15,500																														0
16,000																														0
16,500													1																	1
Total	1	1	4	7	9	16	19	22	29	39	43	40	42	50	59	46	38	26	31	23	26	15	12	5	5	4	4	2	2	634

$r = -0.397 \pm 0.023$

TABLE V.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Jerseys, all ages*

Percentage of fat

Yield of milk, in pounds	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	Total.
4,000																								2	1		2	1	3		1				10
4,500																	1	2	2	2	5	7	5	3	2		2								31
5,000										2	1	1	11	2	12	7	11	9	7	10	9	6	6	1	7	2		1			1	1			108
5,500						1	3	2	6	8	4	7	7	14	17	12	12	9	11	15	9	4	4		5	6	3		3						162
6,000					1	1	2	4	11	8	11	15	13	25	12	21	17	13	10	9	8	6	5	8	5	2	5	2	4						227
6,500					5	2	5	7	6	9	13	22	14	20	19	17	17	13	18	14	8	6	9	2	4	2	1					1			234
7,000			1	1	2	7	10	5	9	12	11	17	29	25	25	28	13	9	16	13	10	14	8	5	4		1	1	1						279
7,500	1	1	1	1	4	5	4	9	9	10	18	14	29	15	10	19	13	21	14	14	12	6	6	3	1	1	2		1						253
8,000			1	1	4	7	3	11	10	14	17	15	13	18	21	15	11	9	11	10	9	6	6	1	1			1							226
8,500			1	3	5	4	5	5	8	16	20	14	15	9	12	17	11	6	5	4	5		2	1	1										169
9,000			1	2	4	5	8	4	9	7	9	16	10	9	8	7	4	1	2	4	4	2	1		1		1							1	123
9,500			1	3		4	10	6	5	7	13	3	8	9	10	4	2	5	5	5	1	1	2	1											105
10,000				1	1	5	4		6	5	9	5		6	5	6	2	3	6	1	1	1	2	1					1						72
10,500	1	1	1		2	1		1	7	5	5	6	3	4	3	1	3	1	2		3	2													52
11,000			2	2	2	1	3	2	2	1	3		2	1	2	3	1		1			1													29
11,500				1	1	2		4	4	1	2	1		3	1				1	1	1														23
12,000						1	2			1		1	1	1						1			1												9
12,500					1			1	1	1	2	2			1												1								10
13,000									1	1				1		1			1			1													6
13,500						1		1																											2
14,000					1	1			1	1	2									1															7
14,500						1		1																											2
15,000										1																									1
15,500																																			0
16,000																																			0
16,500													1																						1
Total	2	2	9	15	33	49	59	63	95	110	140	139	101	166	164	158	174	104	124	104	85	63	57	28	32	13	18	6	13	0	2	2	0	1	2,141

$r = -0.354 \pm 0.013$.

TABLE VI.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Guernseys 2 to 3 years of age*

Percentage of fat

Yield of milk, in pounds	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	5.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	Total.
4,500																							1			1			1			3
5,000														2	1	5	6	1	7	6					2	2		1		1		34
5,500											4	2	6	7	8	4	8	11	6	4	3	4	6	4	2	3		1	1			84
6,000						1	2	1	4	3	7	13	12	9	23	15	10	19	18	8	4	9	5	2						1	1	167
6,500				1	1	4	1	6	7	10	10	14	23	15	12	15	10	7	8	6	9	5	1	1	1		2					169
7,000				1	1	5	8	4	10	13	13	11	14	21	10	15	9	14	11	9	6	2		4	2	3			1			187
7,500					1	2	7	4	10	11	19	16	11	14	16	11	11	11	17	5	2	3	1	1			1					174
8,000					2	6	7	6	6	6	12	9	11	14	23	10	12	8	5	6	2	1	1	1								148
8,500				1		3	2	6	8	3	5	11	9	9	8	10	7	9	4	3			2	1		1						102
9,000					5	1	2	3	5	11	9	11	6	8	8	12	4	3	2	2	2	2	2									98
9,500	1				3	1	4	3		7	4	5	3	1	6	6	6	5	2	4	5	2										68
10,000							1	1	4	3	5	5	6	4	3	2	2	5	3		1		1									46
10,500						1	2	4	2	6	2	2	1	1	2	4	5	4	1		2					1						40
11,000					1		1			1	3	1	2	1	2	2	2	1		1		1										19
11,500									2	2	1	1	1	1			3				2											13
12,000							1			1	1		1			1		1	1	1												8
12,500										1	1						1			1												4
13,000		1			1							1		1	1	1																6
13,500														1																		1
14,000														1																		1
14,500																																0
15,000					1								1		1																	3
Total	1	1	0	3	16	24	38	38	58	73	96	102	107	110	124	113	96	99	85	56	38	29	20	14	7	11	3	2	3	2	1	1,375

$r = -0.251 \pm 0.017$

TABLE VII.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Guernseys 3 to 4 years of age*

Percentage of fat

Yield of milk, in pounds	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	Total.
5,000																				1					1							2
5,500																	2	1	1	2	1	2	7	1								17
6,000										1			4	4	5	6	1	2	4	2	1	1	1	1		2					1	36
6,500								1		3	3	7	11	11	10	9	4	6	4	3	1	1		2				2				78
7,000						2	1	3	3	8	4	6	2	4	9	2	5	2	5	3	3	2	3			1	1		1			70
7,500		1		2	2	2	1	2	2	5	6	8	8	12	6	8	5	3	4	1	2		1		1	1						83
8,000			2		2	3	1	8	8	4	6	9	4	7	2	4	2	5	3	3			1									74
8,500				1	1	2	1	3	2	6	6	7	6	3	1	7		4	3	1	1	3	1			1						60
9,000				1		1	2	1	2	3	6	2	5	4	6	2	4	1	1	1	2											44
9,500			1		1	3	6	6	4	3	7	6	6	7	2	4	4	5		1							1					67
10,000	1				3		1	2	3	1	1	2	5	2	3	1	1	3														29
10,500				1		1		2	4	2	1	1	3	1	2	1		2														21
11,000				1		1	2			2	2	2	3	2	2			1					1									19
11,500									2	2	2	2		1		1	1			1												12
12,000								2	1		1	1		1		2	1															9
12,500							1	1	1	1		1		2			1			1				1								10
13,000						1												1														2
13,500								1																								1
14,000												1																				1
14,500											1		1		1							1										4
15,000												1	1																			2
15,500																																0
16,000												1				1																2
16,500																																0
17,000																																0
17,500																																0
18,000																																0
18,500												1																				1
Total.	1	1	3	6	9	16	16	32	32	41	46	58	59	61	49	48	31	36	25	20	11	10	15	5	2	5	2	2	1	0	1	644

$r = -0.289 \pm 0.024$

TABLE VIII.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Guernseys 4 to 5 years of age*

Percentage of fat

Yield of milk, in pounds	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	Total.
5,500																							1	1									2
6,000																	1			2	2				1								6
6,500															3	4	2	4	2	1	1	2											19
7,000									1	1	2	4	7	3	5	4	3	3	5	1	2												41
7,500									1	6	3	4	5	3	5	4	4	2	4	1	1	1	1										45
8,000					1	3		2	4	7	2	1	6	3	2	5	2	3	1	3	2												47
8,500					1	3	2	4	6	5	3	5	6	5	9	4	5	4	3		2	3											70
9,000	1				3	1	1	5	4	3	5	5	5	1	4	6	7	4	1	1			1	1									59
9,500		1		1		2	3	3	4	1	1	2	2	3	4	4	3	1	3			1	1				1					1	42
10,000				1	2	2		2		2	2	2	2	1		2		2															20
10,500					1	2	2	3	1	1	2	2		1	3	1	2	2	1	2													26
11,000			1		1		2	2	3	4	1	4	1	1			2	1	1	1													25
11,500				1		2		2	1	2	3	1	2	2	2	1	1			1	1												22
12,000								2	1	1	2		1	1	1		4	2															15
12,500							1		2	1			1	1	1			2															9
13,000							1	1				1						1															4
13,500							1			1	1	2	1		2			1			1												10
14,000					1									1																			2
14,500												1	1					1															3
15,000			1			1	1		1			1						1															6
15,500													1																				1
16,000							1									1																	2
16,500																																	0
17,000																																	1
17,500																1																	1
Total	1	1	2	3	10	16	15	26	30	35	27	35	41	26	41	37	36	34	21	13	12	7	4	2	1	0	1	0	0	0	0	1	478

$r = -0.264 \pm 0.029$

TABLE IX.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Guernseys 5 years and over*

Percentage of fat

Yield of milk, in pounds	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	Total.
6,000																										1				1
6,500																		1	2	1	3	4	1		1	1			1	15
7,000														1	2	6	4	7	7	5	1	4		1		2				40
7,500											2	7	3	8	12	10	7	4	7	2	1	1		2	2	1	2			71
8,000								2	5	9	6	8	7	6	12	4	9	8	6	5	3	5	3	2	1					101
8,500							7	7	13	7	5	11	6	13	10	9	12	8	7	4	1	4	2	2	1	1				130
9,000					3	4	3	6	6	3	8	11	13	6	16	4	7	7	3	4	3	3	5		1					116
9,500			2		4	1	6	6	6	6	9	16	9	8	6	10	2	2	4	2		1		1				1		102
10,000	2	1	2	1	1	2	3	2	8	5	13	9	7	14	7	5	4	3		1		1	1					1		93
10,500				1	1	3	2	4	6	15	10	7	7	7	5	7	5	2	4	3	1			1						91
11,000				2	2	4	5	8	8	5	3	8	9	4	10	4	3	1	3		1	1	2						1	84
11,500					1	1	5	5	3	6	6	4	4	2	5	2		1		1										46
12,000			1	2		1	2	2	5	2	4	6	3	2	2	2	4	2	2	1	1									44
12,500			1	1	1	1	2	4	4	6	1	5	1	3	3	1	1	1	1				1							38
13,000					2	2				3	2	2	1	5		1	1	1												20
13,500					1	2	1	1	2	1	2	1	1	1	2			1		1	1									19
14,000						1	1	1	1			2		2		2			1											11
14,500							1	2		1			2						1						1					8
15,000				1				2	1	1	2	1		1	1	2		1		1										14
15,500						2	1			1		1	1		1		1													8
16,000							1	1	3											1										6
16,500						1	1							1						1										4
17,000																														0
17,500												1																		1
18,000													1																	1
18,500																1														1
19,000											1																			1
19,500																			1											1
Total	3	1	6	8	16	25	41	53	71	71	74	100	75	84	94	70	60	50	49	33	16	24	15	9	7	6	2	2	2	1,067

$r = -0.337 \pm 0.018$.

TABLE X.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Guernseys, all ages*

Yield of milk, in pounds	Percentage of fat																																	
	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	5.8	5.9	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	Total
4,500																							1			1			1					3
5,000														2	1	5	6	1	7	6	1				2	3		1		1				36
5,500											4	2	6	7	8	4	8	13	7	5	5	5	8	12	4	3		1	1					103
6,000						1	2	1	4	3	8	13	12	13	27	20	16	21	20	12	8	12	6	3	1	2	2			1	1	1		210
6,500				1	1	4	1	6	8	10	13	17	30	26	23	28	23	14	20	13	16	11	5	1	4	1	2		3					281
7,000				1	1	5	10	5	13	17	22	17	24	31	19	35	19	29	23	24	11	11	2	8	2	5	1	1	1	1				338
7,500			1		3	4	9	5	12	14	32	32	26	35	43	32	30	24	29	15	5	7	2	5	2	2	4							373
8,000				2	2	9	13	9	21	27	29	25	28	30	45	18	30	20	19	15	11	8	4	4	1									370
8,500				1	1	5	14	16	28	18	21	31	27	34	26	29	30	22	19	13	2	7	10	4	1	2	1							362
9,000		1			9	8	7	12	17	20	23	33	26	24	29	26	19	21	10	8	7	7	7	1	2									317
9,500	1		3	1	8	3	15	18	15	21	17	29	20	17	22	22	16	14	12	9	6	3	1	2				3					1	279
10,000	2	2	2	1	2	7	6	4	16	11	21	17	17	25	13	10	9	9	8	1	1	1	2					1						188
10,500				1	2	5	7	10	13	26	15	12	11	11	9	16	12	8	9	4	5			1		1								178
11,000				3	4	5	7	12	10	9	12	12	17	9	15	8	5	4	5	2	2	2	2	1					1					147
11,500					2	1	7	5	7	11	11	10	8	5	8	4	5	3		1	4	1												93
12,000			1	2		1	3	2	9	5	6	9	5	3	4	4	6	8	5	2	1													76
12,500			1	1	1	1	2	6	5	10	4	5	2	4	6	2	2	2	3	1	1		1		1									61
13,000		1			3	2	1	1	1	3	2	3	2	6	1	2	1	1	2															32
13,500	1				1	2	1	2	3	1	3	2	3	3	2	2		1	1	1	1	1												31
14,000						2	1	1	1			2	1	3	1	2			1															15
14,500							1	2		1		1	3	2		1			2				1		1									15
15,000				2	1		1	3	1	2	2	1	3	2	2	2		1	1	1														25
15,500						2	1			1		1	1	1	1		1																	9
16,000							1	2	3				1				2			1														10
16,500						1	1							1						1														4
17,000										1																								1
17,500												1					1																	2
18,000													1																					1
18,500													1			1																		2
19,000											1																							1
19,500																			1															1
Total	4	4	8	16	41	68	111	122	187	211	246	275	275	294	305	273	241	216	204	135	87	76	52	42	21	20	10	7	7	3	1	1	1	3,564

$r = -0.296 \pm 0.010$

TABLE XI.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Holstein-Friesians 2 to 3 years of age*

Yield of milk, in pounds	Percentage of fat: 2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	Total.
6,000	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	1
6,500	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	1
7,000	...	...	...	...	...	...	...	...	...	...	1	1	2	...	...	...	...	...	...	4
7,500	...	...	...	...	...	...	...	...	...	2	3	2	...	2	...	2	...	...	1	12
8,000	...	...	...	...	...	...	1	...	3	2	4	1	2	...	...	...	...	...	...	13
8,500	...	...	...	...	...	...	4	4	5	1	5	2	3	3	1	...	...	...	...	28
9,000	...	...	...	1	1	2	2	4	4	1	7	7	2	2	...	...	...	...	...	33
9,500	...	...	...	2	1	3	1	2	5	3	4	2	...	1	1	1	...	...	...	26
10,000	...	...	...	...	2	2	6	7	5	6	3	4	2	...	...	1	...	...	...	38
10,500	...	...	...	...	...	...	5	4	3	2	4	4	2	3	...	...	1	...	...	28
11,000	...	...	...	...	3	3	3	3	5	3	5	4	1	1	1	...	...	...	...	32
11,500	...	...	1	1	4	3	7	6	5	11	5	5	2	2	...	...	...	...	1	53
12,000	...	...	...	1	1	2	4	5	8	5	4	4	1	1	...	1	...	...	...	37
12,500	...	...	...	1	...	5	3	5	6	3	5	2	3	...	1	2	1	...	1	38
13,000	...	1	...	4	4	1	5	5	6	4	4	6	...	1	...	...	...	...	...	41
13,500	...	...	...	1	...	3	1	...	7	6	3	4	2	1	...	...	1	1	...	30
14,000	...	...	...	...	2	2	2	3	3	3	5	4	2	...	...	1	1	1	...	29
14,500	...	1	...	...	...	3	6	3	...	2	4	1	1	2	1	...	...	...	...	24
15,000	...	...	...	1	3	3	1	2	2	7	4	2	...	2	...	...	1	...	...	28
15,500	...	...	1	...	...	2	4	8	7	2	3	1	1	1	...	...	...	...	...	30
16,000	...	...	...	...	1	2	2	4	1	2	1	1	1	1	1	2	...	...	...	19
16,500	...	...	...	...	...	...	4	1	3	4	2	2	...	...	1	...	1	...	...	18
17,000	...	...	...	1	...	1	...	1	1	2	2	...	1	...	...	...	...	...	...	9
17,500	...	...	...	...	...	1	1	1	...	2	3	...	...	...	...	...	...	...	...	8
18,000	...	...	1	...	1	1	1	...	2	...	1	...	1	...	1	...	1	...	...	10
18,500	...	...	...	...	1	...	...	...	2	...	...	1	...	...	...	...	...	...	...	4
19,000	1	...	...	1	...	1	...	...	1	1	...	...	...	...	...	...	...	...	...	5
19,500	...	...	...	...	...	...	...	...	...	1	...	1	...	1	...	...	...	...	...	3
20,000	...	...	...	...	...	...	...	...	1	...	1	...	...	...	...	...	...	...	...	2
20,500	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	1
21,000	...	...	...	...	...	1	...	...	...	1	...	...	1	...	...	...	...	...	...	3
21,500	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
22,000	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	1
22,500	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
23,000	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	1
Total..	1	2	3	14	24	42	63	68	85	77	83	62	30	25	9	10	7	2	3	610

$r = -0.116 \pm 0.027$.

TABLE XII.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Holstein-Friesians 3 to 4 years of age*

Percentage of fat

Yield of milk in pounds	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	Total.
8,000												1	1								2
8,500										1	1		1								3
9,000							1	1	1	2	2	1	4								12
9,500								2	1	1	2	1		1	2					1	12
10,000							1		2	3	4	1	1								12
10,500						1	2		2		6	2	3								16
11,000			1			1	4	3			1	1	3	2							16
11,500				1	1	2	2	2	4	1	1	1	2	1							18
12,000			1	1	1	2	1	2	4	2		2	1	1							18
12,500					1		1	4	4	1	2	2			1			1	1		18
13,000			1	1	1	2	1	1	5	2	1	2	1	1		1					20
13,500	1			2	1	4	5	3	3	3	7	2	3	1	1						36
14,000		1	1	1		2	3	2	2	2		1	2				1				18
14,500	1				2		2	5	2	4	2	1	1	2							22
15,000			1			3		2	3	2	2		1								14
15,500					2	1		1	3		3			1	2	2					15
16,000				1	2	2	3		3	2	1						1				15
16,500						2		5	1		2	2									12
17,000						2	1			2			1								6
17,500				1	2	2		1	5		1		1	1		1					15
18,000								2	1	1	1										5
18,500				1	1		2	1	2				1								8
19,000				1				1	2	2	1							1			8
19,500								1	1	2											4
20,000			1		1			2													4
20,500				1																	1
21,000									1		1										2
21,500						1				1											2
22,000																					0
22,500		1						1						1							3
23,000							1								1						2
23,500																					0
24,000					1																1
24,500																				1	1
Total	2	2	6	11	16	27	30	42	52	34	42	20	27	12	7	4	2	2	1	2	341

$r = -0.160 \pm 0.036$

TABLE XIII.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Holstein-Friesians 4 to 5 years of age*

Percentage of fat

Yield of milk in pounds	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	Total.
8,000															1							1
8,500													1									1
9,000														1	1							2
9,500									1		1				2	1						5
10,000													1		1	1						3
10,500							2	1	2	1	2	1										9
11,000					1	1	4	1	3	2	2											14
11,500					1	1	4		1	5	1			1								14
12,000					1	3	2		5	2	2	1		2		1						19
12,500				1	3	3	1	2	3			2	1	1								17
13,000				1		1	1	1	4	3	1		1									13
13,500				1	1	2	3	2	5	2	3		1	1								21
14,000				1	1	1	1	3	5	1	5	2										20
15,500					1	2		1		6		2	2		2							16
15,000					1	1	3	2	2		1	3	1	2								16
15,500	1	1			1	1	2	3	1	1	3		1	1								16
16,000					3		3	3		4	2											15
16,500					1		4	1				2		1	1							10
17,000				1		1	3	5	2	3	1	4	1									21
17,500			1	1	2	1		1	2	1	1		1						1			12
18,000				1	1					1	1											4
18,500				2			1	1	2		2	1		1								10
19,000	1					1		1		1		1										5
19,500				1			2	2		1												6
20,000									1	2		1	1									5
20,500											1				2				1		1	5
21,000										1												1
21,500					1					1												2
22,000							1	1			1											3
22,500								1						1								2
23,000																						0
23,500																						0
24,000		1		1				1		1												4
Totals	2	2	1	11	19	19	37	33	39	39	30	20	12	12	10	3	0	0	2	0	1	292

$r = -0.088 \pm 0.039$

TABLE XIV.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Holstein-Friesians 5 years and over*

Percentage of fat

Yield of milk, in pounds	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	Total.
9,000	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	1	2
9,500	...	...	...	...	...	...	...	...	...	1	...	...	1	...	1	...	...	...	...	...	...	...	...	...	3
10,000	...	...	...	...	...	...	...	...	...	1	...	...	...	1	...	...	...	...	...	...	...	...	...	...	2
10,500	...	...	...	...	...	...	...	...	1	2	...	1	...	1	...	...	...	...	...	...	...	...	...	...	5
11,000	...	...	...	...	...	...	...	1	4	3	1	2	3	1	...	1	...	...	...	...	...	...	...	...	16
11,500	...	...	...	...	...	1	5	2	3	6	1	2	...	...	...	1	...	...	...	...	...	...	...	...	21
12,000	...	...	...	...	...	...	1	2	6	4	1	3	...	1	...	...	...	...	...	...	...	...	...	...	18
12,500	...	...	...	...	4	6	7	4	4	9	4	4	4	...	...	2	...	...	...	...	...	...	...	...	48
13,000	...	...	...	...	5	6	3	8	6	6	3	1	...	...	1	...	2	...	...	...	...	...	...	...	41
13,500	...	1	...	1	1	3	4	3	7	3	1	5	1	...	...	1	...	...	...	...	...	...	...	...	31
14,000	...	...	...	1	2	1	2	6	6	4	4	7	1	1	...	...	1	1	1	...	...	...	...	...	38
14,500	...	...	...	1	...	3	3	6	3	7	5	4	2	1	...	1	...	...	...	...	...	...	...	...	36
15,000	...	...	1	5	1	1	8	5	2	7	...	3	4	3	...	...	...	1	...	...	...	...	...	...	41
15,500	...	...	...	1	2	1	5	3	4	6	4	3	1	1	1	...	...	...	...	...	...	...	...	...	32
16,000	...	...	...	1	1	3	5	12	3	2	2	4	2	...	1	...	...	...	...	1	...	...	...	...	37
16,500	...	...	1	...	2	8	2	5	7	...	4	2	...	3	...	...	1	...	...	...	...	...	...	...	35
17,000	1	...	...	2	2	5	2	6	9	2	4	1	2	1	...	...	...	...	...	...	...	...	...	...	37
17,500	...	...	1	...	2	1	5	7	5	4	6	1	2	2	...	1	...	...	...	...	...	...	...	...	37
18,000	...	...	...	...	2	5	5	2	2	1	1	5	...	1	1	...	...	2	...	...	...	...	...	...	27
18,500	...	...	1	...	1	1	4	5	4	4	8	...	...	...	...	1	...	...	...	...	...	...	...	...	29
19,000	...	...	...	...	...	5	...	3	5	4	1	...	...	...	...	...	...	...	...	...	...	...	...	...	18
19,500	...	...	...	...	...	2	4	2	5	4	3	...	...	1	...	1	...	...	...	...	...	...	...	...	22
20,000	...	...	1	...	...	1	1	4	3	5	1	...	...	...	...	...	...	...	...	...	...	...	...	...	16
20,500	...	...	1	...	4	1	1	4	...	3	1	...	...	2	...	...	...	...	...	...	...	...	...	...	17
21,000	...	...	...	...	1	1	...	1	...	1	...	...	...	...	...	...	...	1	...	1	...	...	...	...	6
21,500	...	...	...	...	1	1	2	...	4	2	...	1	...	1	...	...	...	...	...	...	1	...	...	...	13
22,000	...	...	...	1	...	...	2	1	3	...	3	...	...	1	1	...	...	...	...	...	...	...	...	...	12
22,500	...	...	...	...	...	2	2	2	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	8
23,000	...	...	...	...	...	...	...	1	1	...	2	...	...	1	...	...	...	...	...	...	...	...	...	...	5
23,500	...	...	...	...	...	1	...	1	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	3
24,000	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
24,500	...	...	...	...	...	...	...	1	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	2
25,000	...	...	...	...	1	1	...	...	...	...	...	...	...	2	...	...	...	...	...	...	...	...	...	...	4
25,500	...	...	...	...	...	...	1	...	...	1	...	...	...	1	1	...	...	...	...	...	...	...	...	...	4
26,000	...	...	...	1	1	...	...	3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	5
26,500	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
27,000	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
27,500	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	1
28,000	...	...	...	...	1	...	...	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3
28,500	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
29,000	...	...	...	...	1	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2
29,500	...	...	...	...	...	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2
30,000	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
30,500	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
Total	1	1	7	14	35	64	74	101	99	93	61	51	23	27	8	9	4	5	1	2	1	0	0	1	682

$r = -0.115 \pm 0.026$

TABLE XV.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Holstein-Friesians, all ages*

Percentage of fat

Yield of milk, in pounds	2.6	2.7	2.8	2.9	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	Total.
6,000	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	1
6,500	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	1
7,000	...	...	...	...	...	...	...	...	...	...	1	1	2	...	...	...	...	...	...	...	...	...	...	...	4
7,500	...	...	...	...	...	...	...	...	...	2	3	2	...	2	...	2	...	...	1	...	...	...	...	...	12
8,000	...	...	...	...	...	...	1	...	3	2	4	2	3	...	1	...	...	...	...	...	...	...	...	...	16
8,500	...	...	...	...	...	...	4	4	5	2	6	2	5	3	1	...	...	...	...	...	...	...	...	...	32
9,000	...	...	...	1	1	2	3	5	5	3	9	8	6	3	2	...	...	...	...	...	...	...	...	1	49
9,500	...	...	...	2	1	3	1	4	7	5	8	3	1	2	6	2	...	...	...	1	...	...	...	...	46
10,000	...	...	...	...	2	2	7	7	7	10	7	5	4	1	1	2	...	...	...	...	...	...	...	...	55
10,500	...	...	...	...	...	1	9	5	8	5	12	8	5	4	...	...	1	...	...	...	...	...	...	...	58
11,000	...	...	1	...	4	5	11	8	12	8	9	7	7	4	1	1	...	...	...	...	...	...	...	...	78
11,500	...	...	1	2	6	7	18	10	13	23	8	8	4	4	...	1	...	...	1	...	...	...	...	...	106
12,000	...	...	1	2	3	7	8	9	23	13	7	10	2	5	...	2	...	...	...	...	...	...	...	...	92
12,500	...	...	...	2	8	14	12	15	17	13	11	10	8	1	2	4	1	1	2	...	...	...	...	...	121
13,000	...	1	1	6	10	10	10	15	21	15	9	9	2	2	1	1	2	...	...	...	...	...	...	...	115
13,500	1	1	...	5	3	12	13	8	22	14	14	11	7	3	1	1	1	1	...	...	...	...	...	...	118
14,000	...	1	1	3	5	6	8	14	16	10	14	14	5	1	...	1	3	2	1	...	...	...	...	...	105
14,500	1	1	...	1	3	8	11	15	5	19	11	8	6	5	3	1	...	...	...	...	...	...	...	...	98
15,000	...	...	2	6	5	8	12	11	9	16	7	8	6	7	...	...	1	1	...	...	...	...	...	...	99
15,500	1	1	1	1	5	5	11	15	15	9	13	4	3	4	3	2	...	...	...	...	...	...	...	...	93
16,000	...	...	...	2	7	7	13	19	7	10	6	5	3	1	2	2	1	...	...	1	...	...	...	...	86
16,500	...	...	1	...	3	10	10	12	11	4	8	8	...	4	2	...	2	...	...	...	...	...	...	...	75
17,000	1	...	...	4	2	9	6	12	12	9	7	5	5	1	...	...	...	...	...	...	...	...	...	...	73
17,500	...	...	2	2	6	5	6	10	12	7	11	1	4	3	...	2	...	...	1	...	...	...	...	...	72
18,000	...	...	1	1	4	6	6	4	5	3	4	5	1	1	2	...	1	2	...	...	...	...	...	...	46
18,500	...	...	1	3	3	1	7	7	10	4	10	2	1	1	...	1	...	...	...	...	...	...	...	...	51
19,000	2	...	...	2	...	7	...	5	8	8	2	1	...	...	...	...	...	1	...	...	...	...	...	...	36
19,500	...	...	...	1	...	2	6	5	6	8	3	1	...	2	...	1	...	...	...	...	...	...	...	...	35
20,000	...	...	2	...	1	1	1	6	5	7	2	1	1	...	...	...	...	...	...	...	...	...	...	...	27
20,500	...	...	1	1	4	1	1	4	...	4	2	...	...	2	2	...	...	...	1	...	1	...	...	...	24
21,000	...	...	...	...	1	2	...	1	1	3	1	...	1	...	...	...	...	1	...	1	...	...	...	...	12
21,500	...	...	...	...	2	2	2	...	4	4	...	1	...	1	...	...	...	...	...	...	1	...	...	...	17
22,000	...	...	...	1	...	...	3	2	3	...	4	1	...	1	1	...	...	...	...	...	...	...	...	...	16
22,500	...	1	...	...	...	2	2	4	1	...	1	...	...	2	...	...	...	...	...	...	...	...	...	...	13
23,000	...	...	...	...	...	1	1	1	1	...	2	...	...	1	1	...	...	...	...	...	...	...	...	...	8
23,500	...	...	...	...	...	1	...	1	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	3
24,000	...	1	...	1	1	1	...	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	6
24,500	...	...	...	...	...	...	...	1	...	...	...	1	...	...	...	...	...	...	...	1	...	...	...	...	3
25,000	...	...	...	...	1	1	...	...	...	...	...	...	...	2	...	...	...	...	...	...	...	...	...	...	4
25,500	...	...	...	...	...	...	1	...	...	1	...	...	...	1	1	...	...	...	...	...	...	...	...	...	4
26,000	...	...	...	1	1	...	...	3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	5
26,500	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
27,000	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
27,500	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	1
28,000	...	...	...	...	1	...	...	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3
28,500	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
29,000	...	...	...	...	1	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2
29,500	...	...	...	...	...	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2
30,000	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
30,500	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
Total	6	7	17	50	94	152	204	244	275	243	216	153	92	76	34	26	13	9	7	4	2	0	0	1	1,925

$r = -0.133 \pm 0.015$

TABLE XVI.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Ayrshires 2 to 3 years of age*

Percentage of fat

Yield of milk, in pounds	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	Total.
5,500						2		1																			3
6,000				2			3	2		1	6		1														15
6,500				1		2	5	5	3	4	5	3	4	1		1											34
7,000			1	5	5	4	12	5	4	14	7	6	3	2		1	1										70
7,500		1		1	2	4	3	3	12	8	9	2	6	1	1		1										54
8,000			1	6	2	1	4	3	7	8	5	3	6	3													49
8,500	1		1	2	1	1	6	4	2	5	3	1	2	3	1	1											34
9,000				1	2	3	6	1	5	2	1	1	1		1												24
9,500				1		2	2	4	2	3	2	2				1	1										20
10,000			1		2	3	2	3	3	3	2	1	1														21
10,500			1	1				1	1	1	1	1															7
11,000						1		2			1	1	1	1												1	8
11,500																											0
12,000									1		1																2
12,500										1																	1
13,000											1																1
Total	1	1	5	20	14	23	43	34	40	50	44	21	25	11	3	4	3	0	0	0	0	0	0	0	0	1	343

$r = +0.019 \pm 0.036$.

TABLE XVII.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Ayrshires 3 to 4 years of age*

Percentage of fat

Yield of milk, in pounds	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	Total.
6,500									1	1	1	1			1					5
7,000				1			2	1	1	1	1			3						10
7,500							3	2	1	3	5	6	1	2		1				24
8,000						1	3	3	4	2	5	2	2			1				23
8,500					3	1		4	4	3	6	2	3	5	2	2				35
9,000					1	2	1	2	2		1	3	4	1	1	1		1		20
9,500					2	1		1	3	3	3		1	1		1	1			17
10,000				1	1	1		1	2	2	4	2	2	2					1	19
10,500				1	1		3	1		1	1	1	1	1						11
11,000					1			2		2		1				1				7
11,500							1				1		1							3
12,000	1									1					1					3
12,500				1					1				1							3
13,000						1			1	1			1							4
13,500					1															1
14,000										1		1		1						3
14,500								1							1					2
15,000							1			1										2
Total.	1	0	0	4	10	7	14	18	20	22	28	19	17	16	6	7	1	1	1	192

$r = -0.093 \pm 0.048$

TABLE XVIII.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Ayrshires 4 to 5 years of age*

Percentage of fat

Yield of milk, in pounds	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	Total.
7,500								2					1					3
8,000				1			2	3	6	1	3	4	3	3	1			27
8,500				1	1	4	2	2	3	3	1	3			1			21
9,000				2		1	4	5	1	5	3	2	1	1	2	2		29
9,500				4	1	3	3	4	4	3	4	1						27
10,000							2	2	1	3		2			1	1		12
10,500	1		1	1	4	3		1	1	2	1	1						16
11,000							1			1	3	1						6
11,500						1	1	1	1		1							5
12,000											1	1	1					3
12,500										1		1						2
13,000						1			1				1					3
13,500					1		1											2
14,000									1		1							2
14,500																		0
15,000								1										1
15,500																		0
16,000																		0
16,500																		0
17,000																		0
17,500																	1	1
Total	1	0	1	9	7	13	16	21	19	19	18	16	7	4	5	3	1	160

$r = -0.023 \pm 0.053$

TABLE XIX.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Ayrshires 5 years and over*

Percentage of fat

Yield of milk, in pounds	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	Total.
8,500	...	...	...	...	...	...	2	8	4	4	5	...	...	1	...	...	...	...	1	25
9,000	...	...	1	1	...	7	9	10	3	10	3	5	5	5	2	...	...	1	...	62
9,500	...	...	...	3	5	6	9	5	9	4	3	4	6	1	...	1	...	...	1	57
10,000	...	...	1	6	3	3	2	10	10	5	1	1	5	1	...	1	...	1	...	50
10,500	...	1	1	5	2	1	9	7	3	6	5	2	...	...	...	...	...	...	...	42
11,000	...	3	2	4	3	7	6	5	5	4	4	2	1	...	...	...	1	...	...	47
11,500	1	...	1	2	2	4	2	4	3	2	1	4	...	...	1	...	1	...	...	28
12,000	...	...	...	1	2	1	3	4	2	4	1	...	...	...	1	...	1	...	...	20
12,500	...	...	...	...	...	3	3	5	3	3	1	...	...	1	...	...	1	...	...	20
13,000	...	...	1	2	2	...	1	3	...	1	2	...	1	1	...	1	...	...	...	15
13,500	...	...	1	1	...	...	...	1	2	...	...	...	...	...	...	...	...	...	...	5
14,000	...	...	...	...	...	1	1	...	...	...	3	...	1	...	...	...	...	...	...	6
14,500	...	...	...	...	1	1	...	1	1	...	...	...	1	...	...	...	...	...	...	5
15,000	...	...	...	...	...	...	...	...	...	...	...	...	1	1	...	...	...	...	...	2
15,500	...	...	...	...	1	...	1	...	1	...	...	1	...	...	...	...	...	...	...	4
16,000	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
16,500	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	1
17,000	...	...	...	...	1	...	...	...	...	...	1	...	...	...	...	...	...	...	...	2
17,500	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
18,000	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	1
18,500	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
19,000	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
19,500	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
20,000	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	1
20,500	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
21,000	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	1
21,500	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
22,000	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	1
22,500	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0
23,000	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	1
Total	1	4	8	25	22	34	49	65	46	45	30	19	21	11	5	3	4	2	2	396

$r = -0.047 \pm 0.034$

TABLE XX.—*Correlation between the percentage of fat in cow's milk and the yield—Registered Ayrshires, all ages*

Percentage of fat

Yield of milk, in pounds	3.0	3.1	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1	5.2	5.3	5.4	5.5	5.6	5.7	Total.
5,500								2		1																			3
6,000						2			3	2		1	6		1														15
6,500						1		2	6	6	4	5	5	3	5	1		1											39
7,000				1	1	5	7	5	13	6	5	14	7	9	3	2		1	1										80
7,500				1		1	5	6	6	6	17	14	10	5	6	2	1		1										81
8,000					2	7	5	6	11	11	13	13	11	6	9	5													99
8,500			1		5	4	5	9	20	14	15	13	9	6	5	6	1	1		1									115
9,000				1	4	3	11	18	23	5	21	11	12	8	8	5	3	1	1										135
9,500					9	8	9	15	14	20	12	10	8	9	1	1	2	1	1	1									121
10,000				2	8	4	5	8	16	16	15	6	7	8	2	1	2		2										102
10,500		1	1	3	8	7	7	10	8	6	10	8	5	2															76
11,000			3	2	5	3	7	10	5	9	5	8	4	2	1	2		1										1	68
11,500		1		1	2	2	6	3	5	4	3	2	5			1		1											26
12,000	1				1	2	1	3	4	3	5	2	2	1	1	1		1											28
12,500				1			3	3	6	3	4	2	2		1			1											36
13,000				1	2	3	1	1	4	2	1	2	2	2	1		1												23
13,500				1	2	1		1	1	2																			8
14,000							1	1		2		5		2															11
14,500						1	1	1	1	1				1	1														7
15,000							1		1	1				1	1														5
15,500						1		1		1			1																4
16,000																													0
16,500								1																					1
17,000						1						1																	2
17,500																		1											1
18,000																1													1
18,500																													0
19,000																													0
19,500																													0
20,000									1																				1
20,500																													0
21,000									1																				1
21,500																													0
22,000											1																		1
22,500																													0
23,000											1																		1
Total	1	2	5	14	49	56	75	106	149	121	132	117	96	65	46	28	10	10	6	2	0	0	0	0	0	0	0	1	1,091

$r = -0.138 \pm 0.020$

TABLE XXI.—*Summary of results from a study of the correlation between percentage of fat and milk yield among different breeds of dairy cows*

Breed and age.	Number of animals.	Milk.			Fat.			Correlation between percentages of fat and yield of milk.
		Mean.	Standard deviation.	Coefficient of variability.	Mean.	Standard deviation.	Coefficient of variability.	
Jersey, 2 to 3 years	877	6,475.0± 28.9	1,270.5±20.5	19.62±0.33	5.425±0.013	0.517±0.008	9.52±0.15	−0.360±0.020
Jersey, 3 to 4 years	411	7,325.0± 45.8	1,377.5±32.4	18.81± .46	5.401± .019	.562± .013	10.41± .25	− .437± .027
Jersey, 4 to 5 years	219	8,043.4± 60.7	1,332.5±42.9	16.57± .55	5.462± .024	.533± .017	9.76± .32	− .359± .040
Jersey, 5 years and over	634	8,814.5± 43.1	1,608.5±30.5	18.25± .35	5.322± .013	.502± .010	9.44± .18	− .397± .023
Jersey, all ages	2,141	7,491.4± 25.0	1,718.0±17.7	22.93± .25	5.392± .008	.525± .005	9.74± .10	− .354± .013
Guernsey, 2 to 3 years	1,375	7,608.0± 28.8	1,584.0±20.4	20.82± .77	5.065± .008	.458± .006	9.03± .32	− .251± .017
Guernsey, 3 to 4 years	644	8,317.0± 49.3	1,854.0±34.8	22.29± .44	5.080± .013	.477± .009	9.39± .18	− .289± .024
Guernsey, 4 to 5 years	478	9,247.0± 62.6	2,030.0±44.3	21.95± .50	5.046± .014	.462± .010	9.13± .20	− .264± .029
Guernsey, 5 years and over	1,067	9,893.0± 42.8	2,067.5±30.2	20.90± .68	4.956± .010	.478± .007	9.64± .30	− .337± .018
Guernsey, all ages	3,564	8,644.4± 23.7	2,095.4±16.7	24.24± .20	5.033± .005	.471± .004	9.35± .08	− .296± .010
Holstein-Friesian, 2 to 3 years	610	12,488.5± 80.4	2,942.0±56.8	23.56± .48	3.462± .008	.294± .006	8.50± .16	− .116± .027
Holstein-Friesian, 3 to 4 years	341	13,938.5±116.5	3,188.0±82.4	22.87± .62	3.433± .012	.322± .008	9.38± .24	− .160± .036
Holstein-Friesian, 4 to 5 years	292	14,825.5±124.4	3,151.0±87.9	21.25± .62	3.417± .012	.313± .009	9.17± .26	− .088± .039
Holstein-Friesian, 5 years and over	682	16,280.0± 94.4	3,654.5±66.7	22.45± .43	3.420± .008	.301± .006	8.81± .16	− .115± .026
Holstein-Friesian, all ages	1,925	14,443.1± 56.0	3,640.7±39.6	25.21± .29	3.435± .005	.305± .003	8.88± .10	− .133± .015
Ayrshire, 2 to 3 years	343	7,960.5± 47.9	1,314.5±33.9	16.51± .44	4.016± .011	.310± .008	7.71± .20	+ .019± .036
Ayrshire, 3 to 4 years	192	9,179.5± 86.1	1,769.5±60.9	19.28± .69	3.936± .014	.314± .011	6.35± .28	− .093± .048
Ayrshire, 4 to 5 years	160	9,584.5± 83.5	1,566.5±59.1	16.34± .63	3.919± .016	.303± .011	7.73± .29	− .023± .053
Ayrshire, 5 years and over	396	10,726.0± 67.1	1,980.0±47.5	18.46± .46	3.865± .011	.313± .008	8.10± .19	− .047± .034
Ayrshire, all ages	1,091	9,417.1± 41.7	2,044.4±29.5	21.71± .33	3.933± .006	.318± .005	8.08± .12	− .138± .020

CONTRIBUTION TO THE KNOWLEDGE OF TOXOPTERA GRAMINUM IN THE SOUTH

By PHILIP LUGINBILL, *Entomological Assistant*, and A. H. BEYER, *Scientific Assistant, Cereal and Forage Insect Investigations, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

Although the spring grain aphis (*Toxoptera graminum* Rondani) has been treated fully in a bulletin [1] issued at a comparatively recent date by the United States Bureau of Entomology, the life-history studies mentioned therein were conducted primarily in the North, where sexual forms appear and the viviparous ones die off annually in the fall of the year. Comparatively little was known at the time of that publication of the life history of the species in the South, especially in the Southeast. Consequently the establishment of a field station at Columbia, S. C., afforded the senior writer a good opportunity to make a study of the insect in that section of the United States. This study was begun in the spring of 1913, continued through the year 1914, and to the spring of 1915. Its purpose was to ascertain whether or not *Toxoptera graminum* in that latitude breeds viviparously throughout the year, and, if so, (*a*) for how long a period it breeds in this manner, (*b*) whether or not the strain becomes weaker as it gets older, and (*c*) whether or not sexual forms are produced.

In addition to this study a number of molting experiments were conducted during the spring and summer months of 1914 in order that the variations in the duration of instars as caused primarily by temperature conditions might be learned.

During the year 1914 the senior writer was assisted in conducting the experiments by the junior writer, and during the former's absence in the fall of that year the latter took charge of the work.

METHOD OF PROCEDURE

The breeding of series of generations began in March, 1913, with an individual taken from the field. The first-born of this individual was isolated as was the first-born of this and each succeeding generation in this series. A careful record was made of their progeny and of the length of life of the individuals constituting the generations. Similarly, a specimen and a record were kept of the last-born of the individual collected, and of the last-born of every succeeding generation originating in this series. The series collectively will be known in this paper as the "A" series.

[1] WEBSTER, F. M., and PHILLIPS, W. J. THE SPRING GRAIN APHIS OR "GREEN BUG." U. S. Dept. Agr. Bur. Ent. Bul. 110, 153 p., 9 pl., 48 fig., 5 diagr. 1912.

Journal of Agricultural Research, Vol. XIV, No. 2
Washington, D. C. July 8, 1918
De Key No. K-68

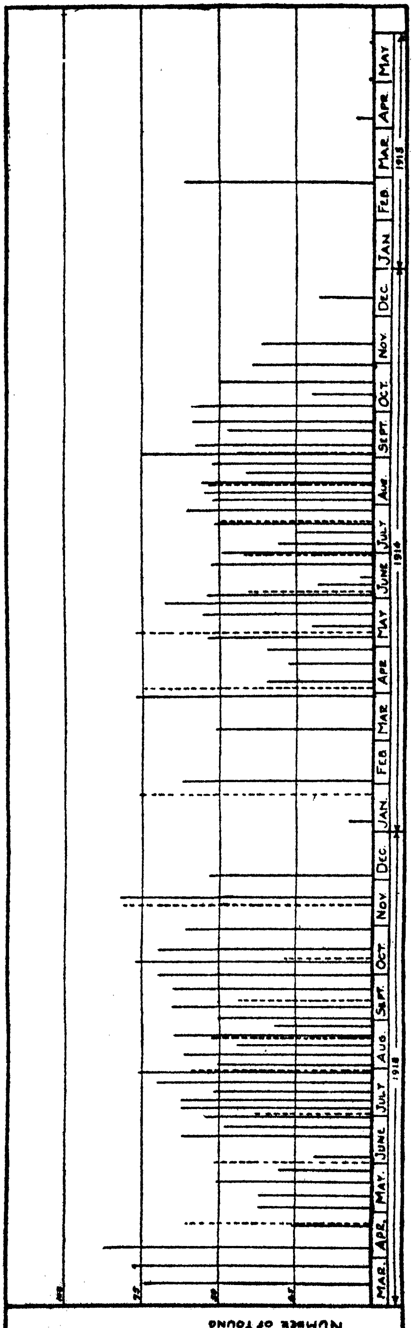

FIG. 1.—Graph showing comparative number of young of the individuals of *Toxoptera graminum* in the A series plotted against the date of birth of the individuals. Columbia, S. C., March 14, 1913, to June 9, 1915. Solid lines represent the young of the first-born and broken lines the young of the last-born individuals.

In the spring of 1914, after the A series had been running for one year, fresh series of first-born and last-born individuals were begun from an individual taken from the field, and similar records made as to the length of life and number of young of the individuals constituting the generations. These series, called the "B" series, were continued until the spring of 1915, or almost one year, after which they were discontinued and other fresh series started similar to the B series of the preceding year. These series, known as the C series, were continued until the A series gave out completely.

The purpose of conducting the B series and C series was to solve part "b" of the problem by comparing results with those obtained in the A series.

APPEARANCE OF OVIPAROUS FORMS

No oviparous forms appeared during the first year the experiments were in progress, the species breeding viviparously throughout that winter (1913–14).

In the fall of the second year, however, all the last-born individuals of the seventeenth generation developed into oviparous females. One individual of the line of generations of those last born in the B series in the late fall of 1914 also

developed into an oviparous female. From one of the oviparous forms in the A series a number of eggs were obtained but these eggs were infertile

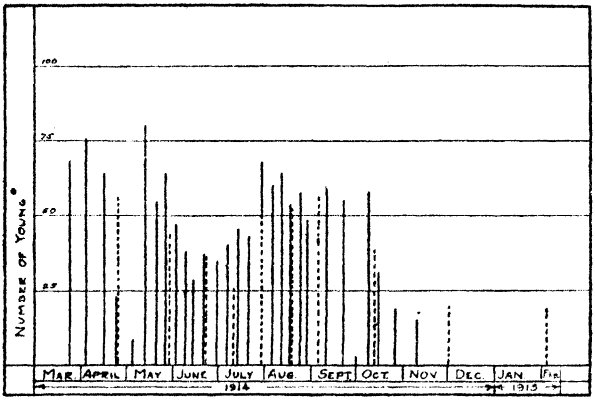

FIG. 2.—Graph showing the comparative number of young of the individuals of *Toxoptera graminum* in the B series plotted against the date of birth of the individuals. Columbia, S. C., March 23, 1914, to April 26, 1915. Solid lines represent the young of the first-born and broken lines the young of the last-born individuals.

as no males had developed in any of the cages and none could be found in nature. The females lived for about one month. It seems entirely probable that if fertile eggs had been obtained they would have hatched either during warm days in winter or early the following spring. According to Webster and Phillips,[1] it is necessary for the eggs to be subjected to cold weather and freezes to make hatching possible. Freezes occur in the latitude of Columbia, S. C., although they are not so severe as are those in the region where the species was studied by Mr. Phillips; yet it seems possible that they were sufficiently severe to meet conditions necessary for the hatching of these eggs.

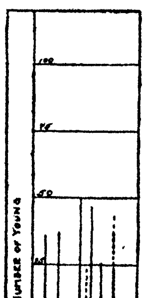

FIG. 3.—Graph showing comparative number of young of the individuals of *Toxoptera graminum* in the C series plotted against the date of birth of the individuals. Columbia, S. C., April 10, 1915, to June 26, 1915. Solid lines represent the young of the first-born and broken lines the young of the last-born individuals.

COMPARATIVE NUMBER OF YOUNG OF INDIVIDUALS IN THE DIFFERENT SERIES

A comparison of the number of young of the individuals in the differents series is shown in figures 1 to 3. The solid lines in these diagrams represent the young of the first-born and the broken lines the young of the last-born individuals. The largest number of young born from any individual in the A series was 88, the reproductive period being only 22 days. This individual was born during the month of April, 1913. During the following winter another individual gave birth to 82 young, the reproductive period in this case

[1] WEBSTER, F. M., and PHILLIPS, W. J. OP. CIT.

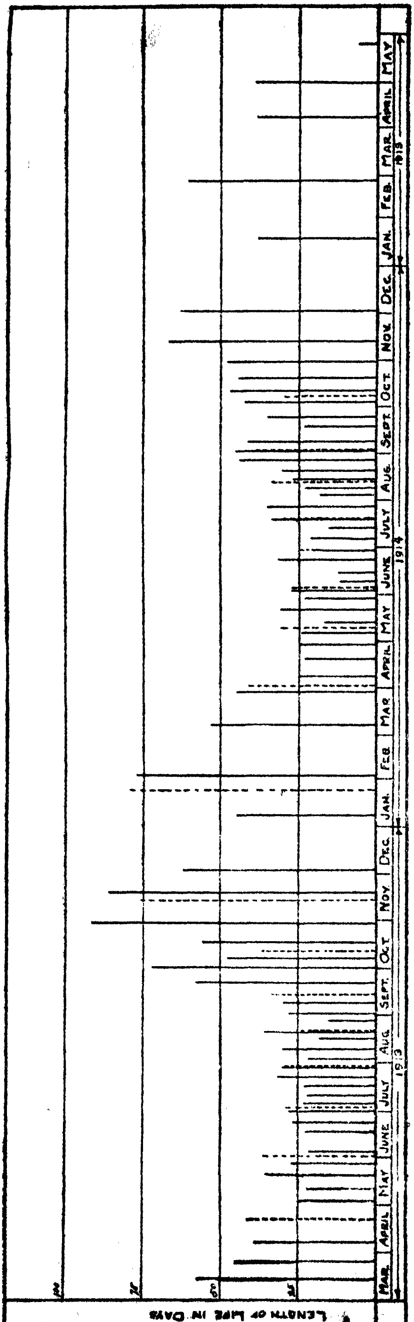

FIG. 4.—Graph showing comparative length of life of individuals of *Toxoptera graminum* in the A series platted against the date of birth of the individuals. Columbia, S. C., March 14, 1913, to June 9, 1915. Solid lines represent first-born and broken lines last-born individuals.

being 60 days. This female was born about the middle of November (1913) and lived until almost the middle of the following February (1914). The low level in reproduction in this series was reached during the summer months. The average number of young produced during these months is only about half that produced in early spring or late fall. Figure 1 shows further that the average number of young of the individuals during the first year was greater than the number produced from the individuals in the second year. This would seem to indicate a weakening of the strain. If, however, the average number of young produced during the second year in the A series is compared with the average number produced in the B series during the same period it will be noted that there were more young in the former series than in the latter. If the number of young aphids during the spring of the third year in the A series be compared with those in the C series during the same period it will be seen that there were more young in the latter series than in the former.

The female representing the seventy-first generation of first-born individuals in the A series died without producing young.

COMPARATIVE LENGTH OF LIFE OF INDIVIDUALS IN THE DIFFERENT SERIES

The length of life of individuals constituting the generations in the different series is clearly represented in figures 4, 5, and 6. In these

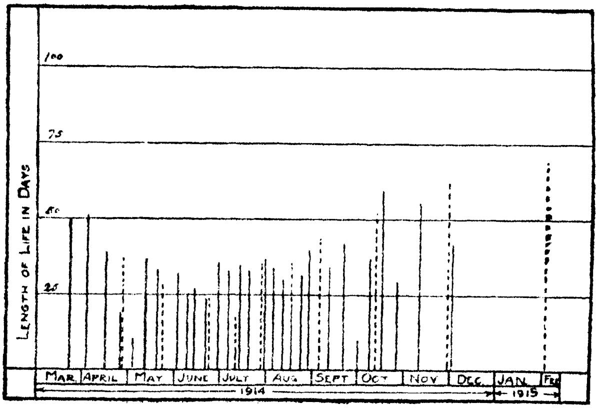

FIG. 5.—Graph showing comparative length of life of the individuals of *Toxoptera graminum* in the B series plotted against the date of birth of the individuals. Columbia, S. C., March 23, 1914, to April 26, 1915. Solid lines represent generations of first-born and broken lines generations of last-born individuals.

diagrams the heavy solid lines represent first-born individuals and the broken ones last-born individuals. By referring to figure 4 it will be seen that the life of individuals born during the months of May, June, July, and August was much shorter than of those born during the following four months; in fact, the individuals born during summer lived only about one-half as long as those born during fall. The longest-lived individual in this series was born during the latter part of October and lived for a period of about three months. The individuals born during the summer months of the second year in the A series were shorter lived than were those born during the same months the preceding year in the same series. Similarly the individuals born during the fall and early winter of 1914 were also shorter lived than were those born during the same period in the preceding year in this series. This fact would seem to indicate that the strain became weaker the second year, and this presumption is supported further by the results obtained from the control experiments conducted during the year 1914, known as the B series. By comparing the individuals in the A series with those of the B series it will be noted

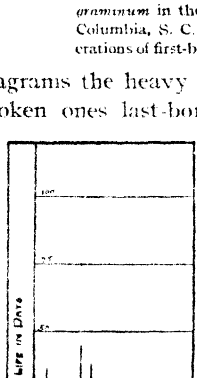

FIG. 6.—Graph showing comparative length of life of individuals of *Toxoptera graminum* in the C series plotted against the date of birth of the individuals. Columbia, S. C., April 10, 1915, to June 26, 1915. Solid lines represent generations of first-born and broken lines generations of last-born individuals.

that the life of those in the former series was shorter than that of those in the latter, the difference, however, not being very great. And if the individuals in the A series during the spring of 1915, the third year of the experiment, are compared with the individuals in the C series it will be noted that the average length of life is also somewhat less in the A series than in the C series. An adequate comparison, however, is difficult here, as there were fewer individuals in the former than in the latter series. In fact, there were only three first-born individuals in the A series as compared with eight first-born in the C series.

NUMBER OF GENERATIONS PER YEAR

A maximum of 33 generations of first-born individuals was obtained in the A series during the first year and a minimum of 9 generations of last-born individuals, making approximately 21 generations for the year. The following year (1914) a maximum of 21 first-born generations was reared and a minimum of 7 generations was reared until September, when the last generation gave rise to oviparous females.

The total number of generations for the whole series, as will be noted by referring to Table I, was 71 of the first-born and 18 of the last-born individuals.[a]

TABLE I.—*First and last born generations, date of birth, reproductive period in days, daily average and total number of young, total length of life, and maximum and minimum temperature during which each generation of Toxoptera graminum lived. Columbia, S. C., 1913–1915*

SERIES A

Generations.			Reproductive period.	Young.		Length of life.	Temperature.	
First-born.	Last-born.	Dates.		Daily average.	Total.		Maximum.	Minimum.
		1913. 1913.	*Days.*			*Days.*	°*F.*	°*F.*
1	....	Mar. 14 to May 11..	28	2.64	74	58	93	42
2	....	26 to 11..	33	2.33	77	46	93	42
3	....	Apr. 7 to 16..	22	4.00	88	39	93	42
4	....	21 to 7..	4	6.50	26	16	93	42
	2	22 to June 2..	32	1.28	61	41	97	42
5	....	May 3 to May 28..	15	2.46	37	25	93	51
6	....	10 to June 2..	14	2.64	37	23	97	51
7	....	19 to 27..	24	2.12	51	39	98	47
8	....	27 to 23..	17	1.36	30	27	98	47
	3	June 1 to July 7..	25	2.08	52	36	100	47
9	....	4 to June 26..	6	3.00	18	22	98	47
10	....	17 to July 10..	15	4.13	62	23	100	65
11	....	23 to 20..	17	2.82	48	27	100	65
12	....	30 to 28..	20	2.75	55	28	102	65
	4	July 2 to Aug. 3..	22	1.72	38	32	102	65
13	....	5 to July 28..	17	3.64	62	23	102	65
14	....	10 to Aug. 1..	15	4.13	62	22	102	69
15	....	16 to 8..	14	3.71	52	23	102	67
16	....	22 to 23..	15	4.66	70	32	96	66
17	....	28 to 27..	16	4.75	76	30	96	61
	5	29 to 27..	16	3.68	59	29	96	61
18	....	Aug. 2 to 24..	12	4.25	51	22	95	61
19	....	8 to Sept. 7..	17	3.58	61	30	95	61
20	....	15 to 2..	13	3.48	44	18	93	61

[a] This table is really a summary of one the writers have prepared showing the date of first and last young, daily production, and maximum and minimum daily temperatures, along with other data given here, but owing to its large size it could not be included with this paper.

TABLE I.—*First and last born generations, date of birth, reproductive period, in days, daily average and total number of young, total length of life, and maximum and minimum temperature during which each generation of Toxoptera graminum lived. Columbia, S. C., 1913–1915*—Continued

SERIES A—continued

Generations.			Reproductive period.	Young.		Length of life.	Temperature.	
First-born.	Last-born.	Dates.		Daily average.	Total.		Maximum.	Minimum.
		1913. 1913.	*Days.*			*Days.*	° *F.*	° *F.*
21	....	Aug. 20 to Sept. 25..	21	3.04	64	36	93	48
	6	21 to 15..	18	2.88	52	25	93	56
22	....	27 to 11..	10	3.20	32	15	93	61
23	....	Sept. 1 to 29..	20	2.50	50	28	92	48
24	....	8 to Oct. 8..	21	3.14	66	30	91	48
	7	13 to 16..	21	2.04	43	33	88	47
25	....	20 to Nov. 17..	32	2.00	64	58	86	26
26	....	29 to Dec. 10..	42	1.66	69	72	86	24
27	....	Oct. 7 to Nov. 24..	46	1.71	77	48	85	28
	8	10 to 15..	26	1.07	28	36	85	26
28	....	16 to Dec. 11..	41	1.68	69	56	78	24
		1914.						
29	....	Oct. 29 to Jan. 30..	52	1.16	61	93	78	24
	9	Nov. 14 to 28..	62	1.30	81	75	78	24
30	....	18 to Feb. 12..	60	1.36	82	86	78	24
31	....	Dec. 3 to 3..	53	1.01	54	62	74	21
		1914.						
32	....	Jan. 7 to Feb. 21..	17	.47	8	45	74	24
	10	24 to Apr. 13..	23	3.30	76	79	78	21
33	....	Feb. 2 to 20..	41	1.51	62	77	84	21
34	....	Mar. 7 to 29..	23	2.26	52	53	92	29
35	....	28 to May 12..	29	3.03	78	45	92	40
	11'	Apr. 3 to 14..	27	2.74	74	41	92	40
36	....	7 to 2..	10	3.50	35	25	92	40
37	....	19 to 12..	8	3.50	28	23	92	40
38	....	28 to 23..	16	2.18	35	25	93	52
39	....	May 6 to 30..	14	3.85	54	24	93	52
	12	9 to June 8..	22	3.50	77	30	96	52
40	....	13 to May 30..	4	5.00	20	17	99	55
41	....	21 to June 21..	13	4.38	56	31	99	56
42	....	20 to 20..	17	4.11	70	23	99	61
43	....	June 2 to 29..	14	3.85	54	27	103	60
	13	5 to July 2..	17	2.35	40	27	103	60
44	....	9 to June 21..	12	1.50	18	12	98	60
45	....	14 to 26..	2	1.50	3	12	103	60
46	....	22 to July 25..	20	2.65	53	33	103	62
47	....	29 to 20..	39	1.25	49	21	98	62
	14	29 to 23..	16	2.62	42	24	98	62
48	....	July 6 to 27..	13	2.38	31	21	102	67
49	....	13 to 28..	7	3.56	25	15	102	61
50	....	19 to Aug. 22..	17	3.05	52	34	102	61
	15	20 to 19..	19	2.57	49	30	102	61
51	....	27 to 31..	17	3.64	62	35	98	68
52	....	Aug. 3 to 21 .	13	4.00	52	18	93	68
53	....	8 to 31 .	15	3.66	55	23	94	69
	16	13 to Sept. 16..	15	3.53	53	34	94	63
54	....	14 to 9..	12	4.66	56	26	94	39
55	....	20 to 19..	23	1.78	41	30	94	69
56	....	27 to Oct. 10..	20	2.60	52	44	94	39
57	....	Sept. 2 to 17..	16	4.56	74	45	94	39
	17	3 to 12..	23	1.86	43	39	94	39
58	....	8 to 19..	17	4.00	68	41	94	33

TABLE I—*First and last born generations, date of birth, reproductive period in days, daily average and total number of young, total length of life, and maximum and minimum temperature during which each generation of Toxoptera graminum lived. Columbia, S. C., 1913-1915*—Continued

SERIES A—continued

Generations.			Reproductive period.	Young.		Length of life.	Temperature.	
First-born.	Last-born.	Dates.		Daily average.	Total.		Maximum.	Minimum.
		1914. 1914.	*Days.*			*Days.*	° *F.*	° *F.*
59	....	Sept. 17 to Oct. 10..	16	2.68	47	23	88	51
60	....	23 to 28..	24	2.45	59	35	86	34
61	....	Oct. 3 to Nov. 14..	28	2.35	66	42	86	34
	[a] 18	8 to 6..	0	.00	0	29	86	34
62	....	10 to 26..	6	3.33	20	47	86	51
63	....	19 to Dec. 2..	32	1.56	50	44	81	22
64	....	30 to 17..	28	1.42	40	48	81	21
		1915.						
65	....	Nov. 12 to Jan. 18..	41	.92	37	67	75	21
66	....	Dec. 2 to Feb. 3..	14	1.28	18	63	65	21
		1915.						
67	....	Jan. 18 to Feb. 26..	1	2.00	2	39	72	27
68	....	Feb. 25 to Apr. 27..	20	3.10	62	61	89	30
69	....	Apr. 7 to May 16..	16	.37	6	39	92	44
70	....	30 to June 9..	1	1.00	1	40	93	56
71	....	May 25 to May 31..	0	.00	0	6	93	58

SERIES B

		1914. 1914.						
1	....	Mar. 23 to May 12..	25	2.72	68	50	92	31
2	....	Apr. 3 to 24..	23	3.30	76	51	96	37
3	....	16 to 25..	23	2.73	63	30	96	40
4	....	26 to 15..	10	2.30	23	19	92	52
	2	27 to June 3..	21	2.66	56	37	96	52
5	....	May 4 to May 15..	2	4.00	8	11	92	52
6	....	12 to June 18..	16	5.00	80	37	99	55
7	....	20 to 21..	25	2.16	54	33	99	55
8	....	26 to 29..	14	4.57	64	34	103	60
	3	29 to 26..	11	4.00	44	28	103	60
9	....	June 2 to July 9..	18	2.60	47	37	103	60
10	....	8 to 3..	8	4.75	38	25	103	60
11	....	13 to 11..	20	1.45	29	28	103	60
12	....	21 to 15..	9	4.11	37	24	103	62
	4	22 to 16..	15	2.40	36	24	103	62
13	....	29 to Aug. 4..	27	1.29	35	36	100	61
14	....	July 6 to 8..	22	1.81	40	33	102	61
	5	11 to July 29..	9	2.88	26	18	102	67
15	....	13 to Aug. 17..	25	1.80	45	35	102	61
16	....	20 to 22..	20	2.15	43	33	102	61
17	....	29 to Sept. 4..	28	2.42	68	37	94	68
	6	29 to 2..	16	3.00	48	35	94	68
18	....	Aug. 5 to 8..	22	2.50	60	34	94	66
19	....	11 to 10..	19	3.36	64	30	94	63
20	....	17 to 8..	15	3.53	53	22	94	63
	7	18 to 22..	13	4.00	52	35	94	39
21	....	24 to 25..	27	2.11	57	32	94	39
22	....	28 to Oct. 7..	26	1.84	48	40	94	39

[a] Oviparous female.

TABLE I.—*First and last born generations, date of birth, reproductive period in days, daily average and total number of young, total length of life, and maximum and minimum temperature during which each generation of Toxoptera graminum lived. Columbia, S. C., 1913–1915*—Continued

SERIES B—continued

Generations.			Reproductive period.	Young.		Length of life.	Temperature.	
First born.	Last born.	Dates.		Daily average.	Total.		Maximum.	Minimum.
		1914. 1914.	*Days.*			*Days.*	° *F.*	° *F.*
	• 8	Sept. 5 to Oct. 19	28	2.00	56	44	94	39
23	......	11 to 15	24	2.54	61	34	88	39
24	......	21 to Nov. 2	34	1.61	55	42	86	40
25	......	30 to Oct. 10	2	1.50	3	10	85	57
26	......	Oct. 8 to Nov. 14	28	2.07	58	37	86	34
	9	12 to Dec. 3	42	.90	38	52	84	22
27	......	15 to 14	41	.76	31	60	81	22
28	......	26 to Nov. 24	14	1.35	19	29	81	22
		1915.						
29	......	Nov. 10 to Jan. 5	24	.66	16	56	70	21
	10	Dec. 1 to Feb. 4	17	1.47	25	65	71	21
30	......	2 to Jan. 13	0	.00	0	42	65	21
		1915.						
	11	Feb. 3 to Apr. 13	26	.73	19	69	83	27

SERIES C

		1915. 1915.						
1	......	Apr. 10 to May 17	22	1.63	36	37	92	44
2	......	20 to 18	19	1.94	37	28	92	54
3	......	28 to 13	7	1.71	12	15	90	56
4	......	May 6 to June 20	25	2.20	55	45	95	56
	2	11 to 2	15	1.60	24	22	93	56
5	......	14 to 21	23	2.04	47	38	95	56
6	......	21 to 21	15	1.73	26	31	95	56
7	......	31 to 30	15	1.60	24	30	95	56
	3	31 to 26	18	2.44	44	26	95	56

VARIATIONS IN DURATION OF INSTARS AS AFFECTED PRIMARILY BY TEMPERATURE CONDITIONS

Temperature conditions play an important rôle in the duration of instars in *Toxoptera graminum.* To ascertain the exact variation in length of instars a series of molting experiments was conducted in 1914 during the months of March, April, May, June, and August. It was intended that a series should be conducted every month for one whole year, but other pressing work prevented this. Enough data, however, have been gathered from the experiments actually carried on to show the positive influence of this factor.

By referring to Table II it will be seen that there was a gradual decrease in the length of individual instars from March to August, the instars in March having been from two to three times as long as those in August.

TABLE II.—*Duration of instars of Toxoptera graminum, Columbia, S. C., 1914*[a]

Born.	Date of first molt.	Period between birth and first molt.	Date of second molt.	Period between first and second molts.	Date of third molt.	Period between second and third molts.	Date of fourth molt.	Period between third and fourth molts.	Entire immature period (approximate).
1914.		*Hours.*		*Hours.*		*Hours.*		*Hours.*	*Hours.*
May 12, 2 p. m.	Mar. 16, 2 p. m.	96	Mar. 20, 2 p. m.	96	Mar. 25, 11 a. m.	117	Mar. 28, 12 NOON	73	382
12, 2 p. m.	16, 2 p. m.	96	23, 2 p. m.	168	27, 2 p. m.	96	29, 12 NOON	46	406
12, 2 p. m.	16, 2 p. m.	96	20, 2 p. m.	96	28, 2 p. m.	192	29, 2 p. m.	24	408
12, 2 p. m.	16, 2 p. m.	96	19, 2 p. m.	72	25, 2 p. m.	144	29, 12 NOON	94	406
12, 2 p. m.	16, 2 p. m.	96	19, 2 p. m.	72	25, 2 p. m.	144	29, 12 NOON	94	406
12, 2 p. m.	16, 2 p. m.	96	23, 2 p. m.	168	27, 2 p. m.	96	29, 12 NOON	46	406
Average		96		112		131.5		62.8+	402.33
Apr. 2, 3 p. m.	Apr. 5, 3 p. m.	72	Apr. 8, 9 a. m.	66	Apr. 12, 12 NOON	99	Apr. 14, 2 p. m.	50	287
2, 3 p. m.	5, 3 p. m.	72	8, 9 a. m.	66	12, 12 NOON	99	14, 2 p. m.	50	287
2, 3 p. m.	6, 2 p. m.	89	8, 5 p. m.	57	13, 8 a. m.	111	15, 2 p. m.	54	311
2, 3 p. m.	6, 8 a. m.	89	8, 2 p. m.	54	12, 12 NOON	94	15, 2 p. m.	74	311
2, 3 p. m.	6, 3 p. m.	96	9, 8 a. m.	65	13, 8 a. m.	96	16, 8 a. m.	92	329
2, 3 p. m.	6, 8 a. m.	89	8, 2 p. m.	54	12, 12 NOON	94	16, 8 a. m.	92	329
Average		84.50		60.33		98.83		65.33	309
May 8, 2 p. m.	May 11, 9 a. m.	67	May 12, 5 p. m.	32	May 14, 12 NOON	43	May 16, 12 NOON	48	190
8, 2 p. m.	11, 9 a. m.	67	12, 5 p. m.	32	14, 12 NOON	43	18, 3 p. m.	99	241
8, 2 p. m.	11, 9 a. m.	67	12, 6 p. m.	32	14, 12 NOON	43	16, 12 NOON	48	190
8, 2 p. m.	11, 9 a. m.	67	12, 5 p. m.	32	14, 12 NOON	43	16, 12 NOON	48	190
8, 2 p. m.	11, 4 p. m.	74	13, 5 p. m.	49	14, 1 p. m.	20	17, 12 NOON	71	214
8, 2 p. m.	11, 11 a. m.	69	Escaped						
Average		68.50		35.40		38.40		62.8	205
June 13, 2 p. m.	June 15, 8 a. m.	42	June 16, 3 p. m.	31	June 18, 3 p. m.	48	June 20, 8 a. m.	41	162
13, 4 p. m.	15, 8 a. m.	40	16, 10 a. m.	26	18, 3 p. m.	53	20, 8 a. m.	41	160
13, 2 p. m.	15, 8 a. m.	42	last						
13, 2 p. m.	15, 8 a. m.	42	16, 10 a. m.	26	18, 5 p. m.	55	20, 8 a. m.	39	162
13, 2 p. m.	14, 5 p. m.	27	16, 10 a. m.	41	17, 4 p. m.	30	20, 8 a. m.	64	162
13, 2 p. m.	15, 8 a. m.	42	16, 3 p. m.	31	19, 8 a. m.	65	20, 3 p. m.	31	169
Average		39.10		31		50.2		43.2	163

Aug. 22, 2 p. m.	Aug. 24, 9 a. m.	43	Aug. 23, 3 p. m.	30	Aug. 26, 5 a. m.	26	Aug. 28, 3 p. m.	46	145
22, 2 p. m.	24, 9 a. m.	43	25, 9 a. m.	24	26, 2 p. m.	29	28, 8 a. m.	42	138
22, 2 p. m.	24, 9 a. m.	43	25, 12 NOON.	27	27, 9 a. m.	45	28, 8 a. m.	23	138
22, 2 p. m.	23, 6 p. m.	28	27, 9 a. m.	87	29, 9 a. m.	48	31, 9 a. m.	48	211
Average.		39.25		42		37.00		39.75	158.00

Mean temperature:	°F.
Mar. 12 to Mar. 29	53.1+
Apr. 2 to Apr. 16	39.8+
May 8 to May 18	70.3+
June 13 to June 20	75.6+
Aug. 22 to Aug. 31	81.1

[a] All references to clock time refer to standard time.

The relation between temperature and immature stages is still more clearly brought out by comparing the average length of the immature stages of each series with the average mean temperature of the period during which each series was conducted. This comparison is well illustrated in figure 7. By referring to this diagram it will be noted that there was a marked shortening in the length of the immature period as the temperature of each month became higher until the month of June was reached. The length of the immature period in August was about the same as that for June when the temperature was becoming higher. The temperature curve and immature-period curve crossed in April. From this we may conclude that the length of the immature period is markedly affected until a certain point is reached, after which a higher temperature apparently has little or no effect in shortening the length of this period.

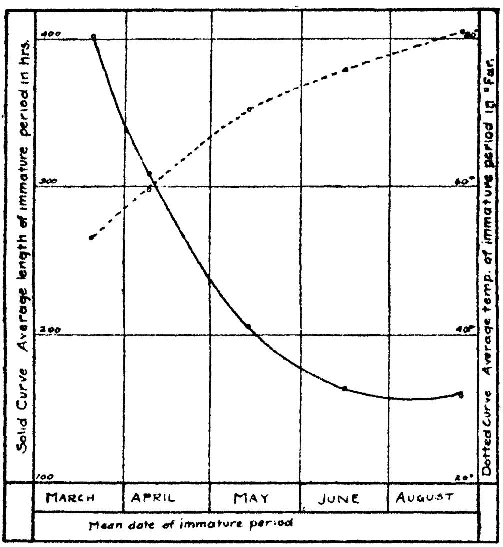

FIG. 7.—Graph showing effects of temperature upon length of immature period of *Toxoptera graminum*, plotted against the mean date of the immature period.

It is quite probable that had the experiments been carried on as originally planned, the immature-period curve would show an upward course after August and the temperature curve a downward course, the two curves crossing later on in the fall of the year.

In figure 8 the length of the immature period of the individuals is plotted against the mean temperature for the period during which the experiments were in progress.

REARING METHODS

In conducting the life-history studies of this species 6-inch flowerpot cages supplied with lantern chimneys covered with cheesecloth were utilized. Oats were used as host plants throughout the year. These cages were all kept under normal conditions throughout the year in an outdoor breeding shelter (Pl. 12, A). The curtains of this shelter were partly raised during sunny days to admit the warm sun's rays. Care was taken, however, that the rays did not strike the chimney covers as

this would have raised the temperature and produced abnormal conditions. At night and during cool, blustery days the curtains were lowered. An interior view of the shelter is shown in Plate 12, B. The arrow on the left of the figure points to the Toxoptera cages.

In conducting molting experiments it was found that a smaller type of cage than that used for line breeding gave best results. Three-inch flowerpots supplied with toy-lantern globes covered over with cheesecloth proved very satisfactory. Owing to the small size of these cages there was less chance for the individuals to get lost. Black fiber paper, such as is used in picture framing, was placed in the bottom of the cages, and this assisted very greatly in detection of the molted skins, which, being whitish, were conspicuous against the dark background.

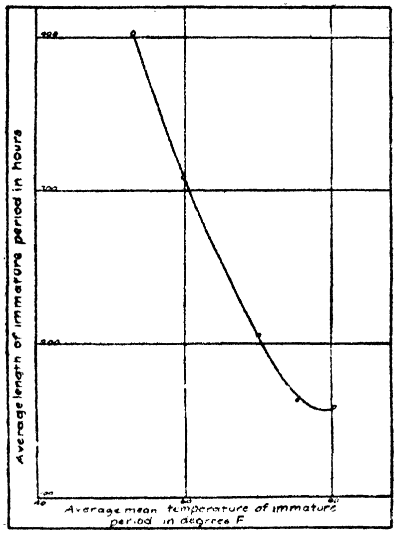

FIG. 8.—Same as figure 7, but in this diagram the average length of the immature period of *Toxoptera graminum* is plotted against the average temperature for that period.

CONCLUSION

Although valuable information on the life history of this species in the South has been obtained from these studies, further work is necessary to prove conclusively all the questions raised. One point has been proved in these investigations, and that is that oviparous forms develop in the latitude of Columbia, S. C. Whether or not the strain becomes weaker as it grows older requires further experimentation before we may be at all certain regarding it. The foregoing experiments would indicate this; yet it was found that insufficient control experiments had been conducted with the main series.

PLATE 12

Toxoptera graminum

A.—Outdoor breeding shelter or insectary at Columbia, S. C., in which the cages were kept throughout the season.

B.—An interior view of the same. The arrow points to the cages used in rearing the insect.

CAN BIOLOGIC FORMS OF STEMRUST ON WHEAT CHANGE RAPIDLY ENOUGH TO INTERFERE WITH BREEDING FOR RUST RESISTANCE?[1]

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COOPERATIVE INVESTIGATIONS BETWEEN THE AGRICULTURAL EXPERIMENT STATION OF THE UNIVERSITY OF MINNESOTA AND THE BUREAU OF PLANT INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE

INTRODUCTION

Doubt has often been expressed whether it is possible to breed cereals permanently resistant to rusts. The opinion is commonly held that either the newly developed resistant variety loses its resistant quality or that the particular rust in question adapts itself to the new variety. The fundamental facts ought to be known if breeding is to be successful.

There are two main possibilities, besides mutation, as far as the rust is concerned. It is possible to assume that a highly resistant variety may occasionally be weakly infected by the rust and that, as a result of its sojourn on the host, the rust acquires additional virulence, thus enabling it to infect the variety with progressively greater ease. If this assumption be true, the degree of virulence of a rust on a particular cereal variety should be directly proportional to the length of its association with that variety, or a physiologically similar one. The second possibility is that the rust may be changed by a closely related host variety, species, or hybrid. That is, assume the presence in a breeding plot, or in a wider area, of varieties completely susceptible (S), moderately susceptible (S—), moderately resistant (R—), and highly resistant (R) to a given rust. The rust from S might not be able to pass directly to R, but might be able to pass to S—, thence to R—, and thence to R. S— and R— would therefore act as intermediaries or bridges between S and R. The rust having once grown on R could then continue to infect R.

If such hypothetical cases as cited above actually occur in nature, the value of breeding for rust resistance would be problematical. It was for this reason that the work reported in this paper was undertaken.

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[2] On leave.

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HISTORICAL REVIEW

Ward (*11*),[1] as a result of his extensive work with biologic forms of the rust of bromes (*Puccinia dispersa* Erikss.), first suggested the possible occurrence of bridging hosts. He suggested that bridging hosts might be hybrids or varieties taxonomically linking one predisposed species with another. Freeman (*4*) obtained results similar to Ward's. Salmon (7) concluded that biologic forms of *Erysiphe graminis* DC. on the genus Bromus might be broken down by the use of bridging hosts. Freeman and Johnson (*5*) and Johnson (*6*) obtained evidence that bridging hosts might also, in some cases, enable a biologic form of *P. graminis* Pers. to infect a host plant which was normally immune. Stakman (*8*) and Stakman and Piemeisel (*9*), on the other hand, could not get any evidence of bridging, nor of rapid changes in the parasitic capabilities of biologic forms of *P. graminis* Pers.

Although Ward first suggested the possible significance of hybrids in connection with bridging, Pole Evans (*3*) first called attention to the actual effect of such hybrids. He found that when immune and susceptible wheats were crossed, the resulting hybrid, whether infected naturally or artificially, was more susceptible to *Puccinia graminis* than the susceptible parent. In addition, he found that the rust from the hybrid was more virulent on the susceptible parent than the rust from that parent itself, and had even acquired the power to infect the immune parent. It therefore acted as a bridge between the susceptible parent and the immune parent, and intensified the action of the rust on the susceptible parent. The significance of these results is obvious, and if they were of universal application the outlook for breeding and successfully growing rust-resistant wheats would be discouraging.

Biffen (*1*) had previously shown resistance in wheats to the yellow-rust *Puccinia glumarum* Erikss. and Henn. to be a recessive Mendelian character and found that the relatively immune forms bred true. After the publication of Pole Evans's results, Biffen (*2*) published the results of extensive experiments, showing that F_2 individuals resulting from crossing susceptible and immune wheats show definite segregation. An analysis of the F_2 plants proved that 25 per cent of the individuals were relatively immune. These bred true, at least from 1907 to 1911. Some of the susceptible forms bred true, while others produced offspring which segregated into immune and susceptible forms in the ratio 1 to 3. Biffen also states that some of the susceptible F_2 plants are more susceptible than the susceptible parent, but that even if plants of the F_1 generation can act as a bridge between the susceptible and immune forms, the effects in the field are negligible. In support of his statement he points to the facts that the immune parents remained practically rust-free for eight seasons, even when growing near F_1 plants of resistant

[1] Reference is made by number (italic) to "Literature cited," p. 122-123.

and susceptible parentage, and that such sharp 3 to 1 segregation could not occur in the F_2 generation if plants with the constitution RR similar to that of the immune parent could be infected by rust from the DR plants. His F_8 resistant plants were still as resistant as those of the F_2 generation. He directs attention to the fact that Rivet wheat, one of the oldest English varieties, is still resistant to *P. glumarum*, and that einkorn, possibly the first wheat to be cultivated, is still resistant to most rusts. He concludes that if varieties lose their immunity, either on account of some change in their own nature or on account of increased virulence of the rust, the change is too slow to affect the work of plant breeders.

EXPERIMENTAL METHODS

In the experiments with *Puccinia graminis tritici-compacti*, unless otherwise specified, the grains used were Haynes Bluestem wheat, (Minnesota 169, *Triticum vulgare*); Manchuria barley (Minnesota 105, *Hordeum vulgare*); Swedish rye (Minnesota 2, *Secale secale*); and Improved Ligowa oats (Minnesota 281, *Avena sativa*). The methods used were similar to those described by Stakman and Piemeisel (*10, p. 431–432*).

In estimating the degree of infection, more significance was attached to the character than to the number of uredinia. The total number of uredinia produced is no adequate index of resistance unless the size be considered together with flecking or yellowing of the leaf. All the inoculated leaves of a highly resistant variety often become infected, but the character of infection is quite different from that in a susceptible variety.

On resistant varieties the uredinia are usually smaller, less confluent, and are usually surrounded by a more or less definite yellowed or whitened area, while the uredinia on susceptible plants produce more spores, become larger, and tend to coalesce. This difference is shown clearly in Plate 14, in which A and B show a susceptible form, while C and D represent a partly resistant variety. The differences between the susceptible Marquis shown at B, Plate 16, and the resistant Kubanka shown at C and D may appear indistinct at first glance; but close observation shows clearly the much more pronounced whitening of the tissues near the uredinia on the partly resistant Kubanka. Usually the uredinia on Marquis are larger than those shown in B, while those on the Kubanka at C and D are about normal. An almost immune form is shown at D and E, Plate 15.

The difference between moderately susceptible and completely susceptible hosts, such as that between club wheats infected with *P. graminis tritici-compacti* and barley infected with the same rust, may be shown in degree of infection only. Barley is infected fairly normally, but not so heavily as club wheats.

The wheat hybrids inoculated with *P. graminis tritici* included F_1 and F_2 generation plants, with those of resistant and susceptible parents for comparison, and in addition F_9 plants of a hybrid which was breeding true for the agronomic characters and showing partial resistance to stem rust. Seedlings of Bobs, one of the parent varieties of the cross described by Pole Evans (*3*), were also tested.

The F_1 plants were of the cross Haynes Bluestem (Minnesota 169; susceptible) × Kubanka (CI[1] 2094; resistant). F_2 plants of two different crosses were used: White Spring emmer (Minnesota 1165; very resistant) × Marquis (susceptible); and Marquis (susceptible) × Kubanka (CI 2094; resistant). The F_9 plants were of the cross Haynes Bluestem (Minnesota 169; susceptible) × Kubanka (CI 2094; resistant). Inoculation methods with seedlings were the same as those already referred to. The F_1 and F_2 hybrid plants, which served as sources for the material used to inoculate seedlings of the parent varieties, were inoculated at time of heading, placed in a large metal moist chamber for two days, then removed to the greenhouse bench.

RESULTS

Attempts were made to change the parasitic capabilities of *P. graminis tritici-compacti*, a new biologic form recently described by Stakman and Piemeisel (*10*), both by the use of what should theoretically be bridging hosts and by confining the rust for a period of time to an uncongenial host. This rust was selected on account of its action within the common-wheat group (*Triticum vulgare*). Most hard spring wheats, such as Haynes Bluestem, Fife (Minnesota 163), and Marquis, as well as many winter wheats of the Crimean group, are resistant, while most soft wheats, such as Early Baart, Dicklow, and Washington Bluestem, are quite susceptible, as are also the club wheats. The rust differs but little from *P. graminis tritici* except in its action on hard wheats of the *T. vulgare* group.[2] A number of grasses, barley, and club wheats are equally congenial hosts for both forms. It would seem that if *P. graminis tritici-compacti* could be induced to transfer normally to resistant hard wheats at all, one of the hosts common to both rust forms should act as an intermediary or bridge between the susceptible grass or wheat and the resistant hard wheat. Barley, which has been shown by Freeman and Johnson (*5, p. 18*) to act as a bridge for *P. graminis* between various cereals, was used in the expectation that it might act as a bridging host in this case also. The results are given in diagram 1.

EXPLANATION OF DIAGRAMS 1 TO 3

In diagrams 1, 2, and 3 wheat is represented by "W," barley by "B," club wheat by "CW," oats by "O," and rye by "R." Transfers are indicated by dashes; thus "W—B" means that the rust was transferred from wheat to barley and to all other cereals indicated in the same vertical column. The results of inoculations are given

[1] CI=Cereal Investigations No. [2] See Stakman and Piemeisel (*10, p. 468–470; Pl. 54, 55*).

in the form of a fraction, the denominator indicating the total number of leaves inoculated and the numerator the number which became infected. The number of leaves which became distinctly flecked but did not develop uredinia, is given after the semicolon. The degree of susceptibility is indicated after each transfer as follows: (s), very susceptible; (s—), moderately susceptible; (r—), moderately resistant; (r), very resistant; (i), immune. Although the word "immune" is used, it is quite likely that oats may be very rarely, indeed, and very weakly infected.

DIAGRAM 1.—Effect of barley on the parasitic capabilities of *Puccinia graminis tritici-compacti*

Puccinia graminis tritici-compacti from *Elymus glaucus.*

- $R\frac{1}{11}$; 7(r)
- $W\frac{16}{17}$(r)—$B\frac{21}{21}$(s—)—
  - $B\frac{54}{54}$(s—)—$W^{a}\frac{31}{31}$(r)—$B\frac{32}{40}$(s—)— . . .
  - $W\frac{62}{68}$; 6(r)
  - $CW\frac{17}{17}$(s)
  - $O\frac{0}{37}$(i)

$B\frac{24}{24}$(s—)— | $B\frac{16}{16}$(s—)— | $B\frac{22}{26}$(s—)— | $B\frac{28}{28}$(s—)— | $B\frac{14}{14}$(s—)— | $B\frac{31}{31}$(s—)— | $B\frac{28}{28}$(s—)

- under $B\frac{16}{16}$: $W\frac{13}{16}$; 3(r) ; $R\frac{2}{8}$; 3(r)
- under $B\frac{22}{26}$: $O\frac{0}{23}$(i)
- under $B\frac{28}{28}$: $W\frac{16}{16}$(r)
- under $B\frac{14}{14}$: $W\frac{10}{14}$(r) ; $R\frac{2}{10}$; 6(r) ; $O\frac{0}{15}$(i)
- under $B\frac{31}{31}$: $W\frac{2}{13}$; 11(r)
- under $B\frac{28}{28}$: $W\frac{6}{22}$; 10(r)

It will be seen from diagram 1 that club wheat and barley are susceptible to the rust, which develops normally on both, although it grows more luxuriantly on club wheat. It is equally clear that barley did not enable the rust to attack Haynes Bluestem wheat any more readily than it could before having been transferred to barley. The rust was confined to barley for seven successive "urediniospore generations" covering a period of four months, and at the end of that time it had not acquired any additional virulence whatever on wheat. The same is true of its action on oats and rye. Neither did it lose any virulence as a result of its sojourn on Marquis wheat on which it developed very weakly. Incidentally the results also showed that the rust did not adapt itself to more luxuriant development on barley, on account of its long association with it. As indicated, the rust does not develop as luxuriantly on barley as on club wheat, and it never acquired that ability. In this case at least barley does not act as an intermediary or bridging host.

[a] Marquis wheat.

57665°—18——4

Club wheat is also a host for both *P. graministritici* and *P. graminis tritici-compacti.* Theoretically, therefore, if bridging species really occur, this host might be expected to act as one on account of its close taxonomic relationship to the common wheats. When the *tritici-compacti* rust was found on club wheat in the field, therefore, inoculations were made on barley and wheat, and finally the rust was confined to club wheat for a period of about four months and transferred to wheat periodically. The results are given in diagram 2.

DIAGRAM 2.—Effect of club wheat on the parasitic capabilities of *Puccinia graminis tritici-compacti*

$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad | \ W^{b}\tfrac{16}{20};\ 3(r)$$

$$CW— \ \Big|\ B\tfrac{7^{a}}{25}(s—)—B\tfrac{39}{39}(s—)— \ \Big|\ B\tfrac{32}{32}(s—)— \ \Big|\ CW\tfrac{34}{34}(s) \ \Big|\ W\tfrac{13}{15}(r)—W\tfrac{7}{10};\ 3(r)$$

$$\qquad \Big|\ W\tfrac{5}{31};\ 1(r) \qquad\qquad \Big|\ W\tfrac{38}{49};\ 7\ (r)— \ \Big|\ CW\tfrac{28}{39}(s)— \ \Big|\ CW\tfrac{21}{21}(s)—CW\tfrac{13}{13}(s)— \ . \ . \ .$$

$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad \Big|\ W\tfrac{11}{21}(r)—CW\tfrac{10}{12}(s)$$

$$CW\tfrac{28}{28}(s)— \ \Big|\ CW\tfrac{81}{81}(s)—CW\ 100\ \text{per cent}(s)—CW\tfrac{35}{35}(s)— \ \Big|\ CW\tfrac{32}{32}(s)— \ \Big|\ CW\tfrac{30}{33}(s)$$

$$\qquad\qquad \Big|\ W\tfrac{8}{10}(r) \qquad\qquad\qquad\qquad\qquad \Big|\ W\tfrac{2}{10};\ 8(r) \ \Big|\ W\tfrac{12}{15}(r)$$

Club wheat did not change the rust at all; neither did the combination of barley and club wheat. As a matter of fact, one would scarcely expect club wheat to change the rust enough to enable it to attack wheat more easily, because it is found on club wheat in the field, and if this host changed the fungus it would not remain different from *P. graminis tritici.*

If neither barley nor club wheat enabled *P. graminis tritici-compacti* to parasitize common wheat more successfully, it would seem possible that some other species of Triticum or, more likely still, some susceptible variety of *T. vulgare* might bring about the desired result. Representatives of the different species of Triticum were inoculated, but none seemed to give particular promise. Some of the soft wheats of the *T. vulgare* group were found to be susceptible and the rust was transferred from them to hard wheats. The results were monotonously similar to those given in diagrams 1 and 2.

After all attempts failed with possible bridging hosts, the effect of successive transfers to wheat itself was next tried. If a closely related variety or species can suddenly and fundamentally change a rust so as to enable it to pass to a normally almost immune variety, it seems reasonable to suppose that, having once established itself on an almost immune

[a] Inoculations made a month after material was collected: consequently few spores germinated.
[b] Fife (Minnesota 163).

host, constant association with this host should increase its virulence. The results of trials to determine this are given in diagram 3.

Diagram 3.—Results of successive transfers of *Puccinia graminis tritici-compacti* to resistant wheat.

P. graminis tritici-compacti from *Hordeum jubatum*.

- *Hordeum jubatum* $\frac{9}{30}$(s)—W$\frac{1}{33}$; 12(r)
- W$\frac{40}{55}$; 15(r)—W$\frac{1}{14}$(r)—W$\frac{1}{2}$(r)[a]
- CW$\frac{36}{36}$(s)
- B$\frac{63}{63}$

P. graminis tritici-compacti from *Elymus glaucus*.

- W$\frac{17}{20}$(r)—W$\frac{34}{36}$(r)—W$\frac{23}{24}$(r)—W$\frac{9}{9}$[b](r)
- R$\frac{4}{27}$(r)—R$\frac{0}{14}$; 8

There was no increase in virulence as a result of keeping the rust on wheat, the character of infection being the same at the end of the experiment as it had been at the beginning.[2]

The effect of hybrids on *P. graminis tritici* was next tried. The results are given in Tables I to V.

Table I.—*Results of inoculations with P. graminis tritici on resistant and susceptible parents with rust from susceptible F_1 hybrid and from stock cultures on susceptible parent*

Source of rust.	Plant inoculated.	Result.	Character of infection.
Stock cultures	Haynes Bluestem	$\frac{50}{50}$	Heavy; numerous, large uredinia.
F_1 hybrid	....do	$\frac{47}{47}$	Do.
Stock cultures	Kubanka 2094	$\frac{49}{49}$	Moderate; uredinia smaller, sharp flecks.
F_1 hybrid	....do	$\frac{47}{47}$	Do.

The F_1 hybrid plant shown in Plate 13, B, furnished the spore material for the inoculations made on the seedling leaves of the parent varieties, Haynes Bluestem (Minnesota 169), and Kubanka (CI 2094). Normal

[a] Uredinium too small to transfer.

[b] For the character of infection of *P. graminis tritici-compacti* on Haynes Bluestem wheat (Minnesota 169) see Stakman and Piemeisel (*10, Pl. 55, A*).

uredinia appeared on leaf blades and sheaths, as well as on the fasciated glumes, and the plant was without doubt susceptible. The two series of inoculations, one from the stock cultures and the other from this hybrid plant, made on seedlings of Haynes Bluestem (Minnesota 169) gave similar results. In both series numerous uredinia appeared in the usual time and developed to normal size. There was, however, no evidence that Bluestem wheat was any more susceptible than when inoculated with rust from other plants of the same variety (Pl. 14, A, B).

Two series of inoculations made on seedlings of the somewhat resistant parent, Kubanka (CI 2094) also failed to reveal any differences in degree of infection resulting from the source of the inoculum. Although uredinia were formed on all leaves inoculated, many of them were smaller than those on the susceptible variety and sharp flecks were nearly always present, indicating at least some resistance. There was no evidence whatever that Kubanka (CI 2094) was more susceptible to the rust from the hybrid than to rust from any other source (Pl. 14, C, D).

TABLE II.—*Results of inoculations with P. graminis tritici on resistant and susceptible parents with rust from susceptible F_2 hybrid and from stock cultures*

Source of rust.	Plant inoculated.	Result.	Character of infection.
Stock cultures....	Marquis............	49/49	Very heavy; large uredinia.
F_2 hybrid........	....do.............	51/51	Do.
Stock cultures....	Emmer (Minn. 1165).	0/54	Nearly immune; no uredinia; very small, light green flecks.
F_2 hybrid........	....do.............	0/61	Do.

Table II shows the results of inoculations with the rust from the F_2 hybrid, White Spring emmer (Minnesota 1165) × Marquis, on seedlings of the two parent varieties, and furnishes the most convincing evidence that this particular susceptible hybrid does not increase the virulence of the rust for either parent variety. Normal uredinia on the hybrid plant shown at A in Plate 15, furnished the spore material used to inoculate the seedlings of susceptible Marquis and the extremely resistant emmer parents. On all the seedlings of Marquis, normal infection resulted, both from inoculations made with rust from stock cultures, and with those from the susceptible hybrid. There was no observable difference in number or size of uredinia. Neither were there any indications of resistance, nor of increased susceptibility in those plants inoculated

with rust from the hybrid (Pl. 15, B, C). A total of 115 seedlings of the remarkably resistant (nearly immune) emmer parent were inoculated, 54 with rust from stock cultures, and 61 with rust from the susceptible hybrid, but not a single uredinium was formed. Very small light-green flecks appeared, but never developed into uredinia. In so far as indicated by these experiments, this emmer may be described as "once resistant, always resistant" (Pl. 15, D, E).

TABLE III.—*Results of inoculations with P. graminis tritici on resistant and susceptible parents with rust from susceptible F_2 hybrid and from stock cultures*

[Marquis × Kubanka (CI 2094)]

Source of rust.	Plant inoculated.	Result.	Character of infection.
Stock cultures....	Marquis............	55 55	Moderately heavy; large, vigorous uredinia.
F_2 hybrid........	.....do.............	47 47	Do.
Stock cultures....	Kubanka (CI 2094).	48 48	Moderate; uredinia smaller, flecks present.
F_2 hybrid........	.....do.............	52 52	Do.

Table III gives the results with the other F_2 hybrid [Marquis × Kubanka (CI 2094)]. The F_2 plant from which urediniospores were used to inoculate seedlings of the two parents, is shown at A in Plate 16. Uredinia are present on leaf, sheaths, and glumes. The results were similar to those previously described (Pl. 16, B, C, D).

Having determined the effect of an F_1 hybrid, and two F_2 hybrids, it seemed advisable to try a hybrid of a later generation which was breeding true for such morphological characters as presence of awns, hairy chaff, durum-like shape of spike, etc. For this work, seed from the F_9 plants of the cross Haynes Bluestem (Minnesota 169) × Kubanka (CI 2094) was chosen. Seedlings were grown, and 66 plants were inoculated with rust from the stock cultures and the resulting urediniospores were used to inoculate seedlings of the two parent varieties. The results are given in Table IV. The inoculations on this hybrid show that it is not yet homozygous for the character of rust resistance, even though it has for several years been breeding true for other characters. Here again, as in previous trials, a sojourn on the hybrid did not increase the virulence of the rust on either parent perceptibly (Pl. 17).

TABLE IV.—*Results of inoculations with P. graminis tritici on resistant and susceptible parents and F_9 hybrid with rust from partially resistant F_9 hybrid and from stock cultures*

Source of rust.	Plant inoculated.	Result.	Character of infection.
Stock cultures....	Hybrid 4 × 38AA....	$\frac{66}{66}$	Variable; uredinia large and numerous on some leaves, small and few on others. Some leaves show sharp flecking, others none.
Do...........	Haynes Bluestem....	$\frac{59}{59}$	Heavy
F_9 hybrid........	.....do.............	$\frac{52}{52}$	Do.
Stock cultures....	Kubanka 2094......	$\frac{55}{55}$	Moderate; uredinia small, flecks present.
F_9 hybrid........	.....do.............	$\frac{49}{49}$	Do.

GENERAL DISCUSSION

The results of the experiments with *P. graminis tritici-compacti* show that barley which both theoretically and from the results obtained by previous investigators might be expected to increase the infection range does not do so. Even susceptible varieties of wheat do not change the parasitic capabilities of the rust so as to enable it to attack a normally resistant variety. Furthermore, the rust does not acquire additional virulence when associated for a long time with a given host. Barley is moderately susceptible to the rust but the relations between host and rust are apparently the same regardless of the length of their association with each other. Wheats resistant to the rust remain resistant regardless of the previous history of the rust.

The results of the work to ascertain the effect of hybrids on *P. graminis tritici* are not at all in agreement with those of Pole Evans (*3*). Bobs, the wheat found to be immune under the conditions of his experiments, is quite susceptible under our conditions, both in the field-rust nursery and greenhouse (Pl. 13, A). Possibly the strain of rust in South Africa is not the same as ours.

On account of the far-reaching significance of Pole Evans's results, the utmost precautions were taken in the present work to detect any differences which might appear in either the resistant or susceptible parents when inoculated with rust from the hybrid as compared with that from stock cultures. In no case, however, was there the slightest evidence of any change in the virulence of the parasite, nor any indication that a short sojourn on a susceptible hybrid had given it any peculiar ability to cause normal infection on a heretofore resistant variety or to cause a more than usually virulent infection on a susceptible variety.

Observations in the rust nursery extending over a period of seven years also serve to strengthen the idea that rust resistance is a definite character present in a greater or less degree in particular varieties and not greatly influenced by cultural conditions. It has not been possible to build up resistance within a susceptible line by continuous selection nor to isolate resistant plants from such a line. Since 1915 several hundred varieties of all degrees of susceptibility and resistance have been grown in the same nursery with hybrids of the F_1, F_2, and later generations and always under optimum conditions for infection—that is, in a severe epiphytotic of stemrust. No instances have been observed of a resistant variety being attacked by a form of stemrust able to cause severe infection, as would be expected if the hybrid plants near by could produce such a rust form. There are seasonal fluctuations in the severity of the rust attack, but the greenhouse experiments here reported, the experimental work in the rust nursery, and the field observations in several States with resistant varieties of spring and winter wheats, and with oats, all point to the conclusion that both rust and host are relatively stable. It seems more likely that resistant varieties will be only of regional value because of the occurrence of different biologic forms in various regions.

There seems, however, no basis, from the facts now at hand, for the fear expressed by Pole Evans that these hybrids once produced will not only gradually lose their own power to resist attacks of the rust, but will also give the parasite new infection capabilities, enabling it to cause greater injury in susceptible varieties and even to attack previously resistant varieties.

SUMMARY

(1) Neither barley nor club wheat enabled *Puccinia graminis tritici-compacti* to attack resistant common wheats or other resistant cereals more vigorously than normally.

(2) *P. graminis tritici-compacti* was confined to barley and resistant wheat for a number of successive generations, but it did not acquire increased virulence for these hosts.

(3) The parasitism of *P. graminis tritici-compacti* was not changed by bridging hosts nor by association with a given host.

(4) *P. graminis tritici* was used to determine the possible action of hybrids as bridging forms. Studies were made of F_1, F_2, and F_9 hybrids in comparison with their resistant and susceptible parent varieties.

(5) Susceptible plants of the F_1 generation of the cross Haynes Bluestem (Minnesota 169) × Kubanka (CI 2094) did not enable the rust to infect seedlings of the resistant parent normally, nor to infect the susceptible parent more virulently.

(6) The culture of stemrust on susceptible plants of the F_2 generation of the cross White Spring emmer (Minnesota 1165) × Marquis had no appreciable effect on the parasite.

(7) Negative results were obtained in attempting to alter the infection capabilities of the rust by growing it for generation on susceptible F_2 plants of the cross Marquis × Kubanka (CI 2094).

(8) F_9 hybrids of the cross Haynes Bluestem × Kubanka (CI 2094) were apparently homozygous for morphological characters but heterozygous for the character of rust resistance. Susceptible hybrid plants did not act as bridges for the rust.

(9) The facts recorded in this paper, supported by experimental work in the rust nursery and by field observations, indicate that rust resistance is comparable with other permanent characters, and that it is not primarily controlled by seasonal conditions, soil type, geographical location, or other cultural conditions. It is rather an hereditary character, which can not be produced by the accumulation of fluctuating variations within a susceptible line, nor broken down by changes in the host or parasite.

(10) The resistance of wheat varieties may vary in different regions because of the presence of different biologic forms of rust.

(11) There seems to be little basis for the belief that hybrids between resistant and susceptible varieties will exert a harmful final effect by increasing the virulence and host range of stemrust.

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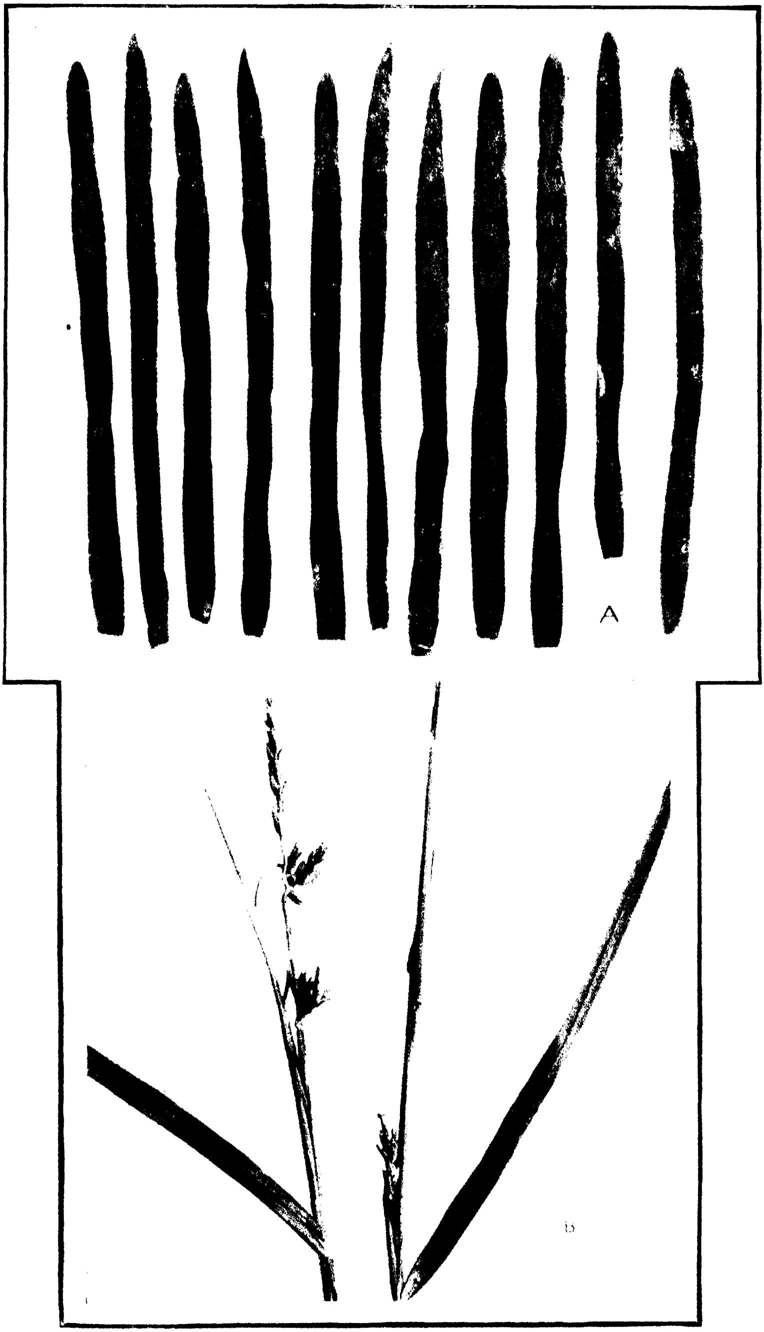
PLATE 13

Puccinia graminis tritici

A.—On seedlings of Bobs wheat (CI 5047).

B.—On susceptible first-generation hybrid, Haynes Bluestem (Minnesota 169) × Kubanka durum wheat (CI 2094).


A
B

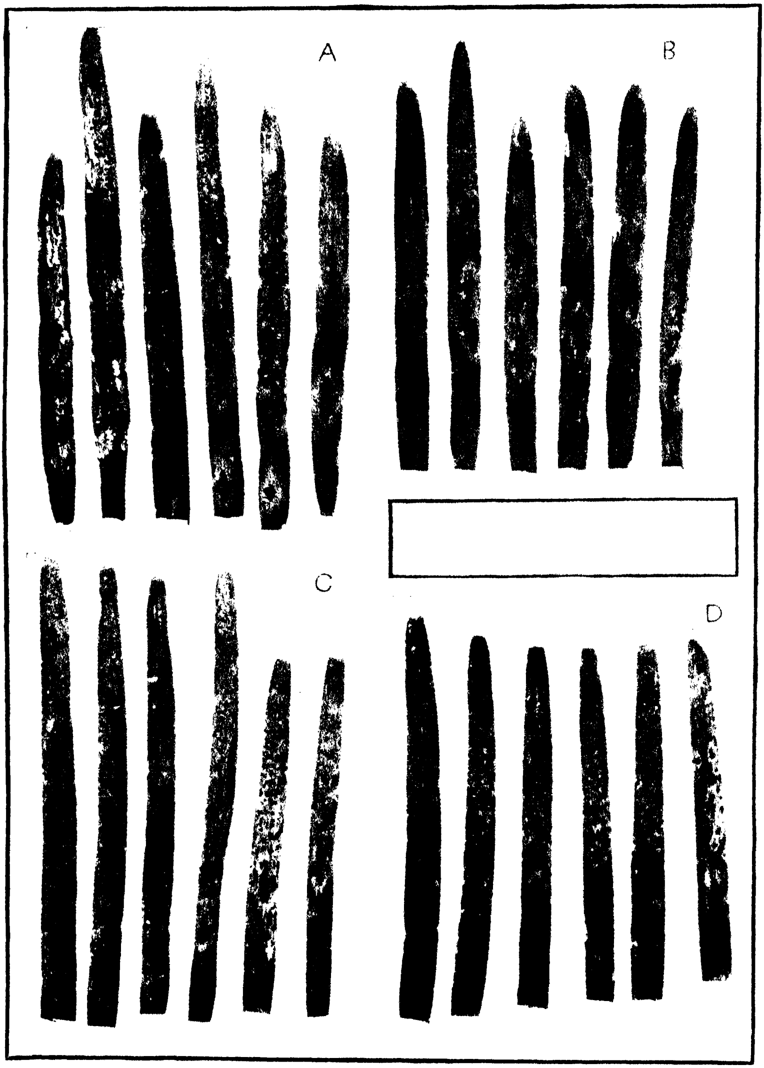

A
B
C
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PLATE 14

Puccinia graminis tritici

A.—On seedlings of Haynes Bluestem inoculated with rust from the susceptible parent—that is, Haynes Bluestem.

B.—On seedlings of Haynes Bluestem, the susceptible parent, inoculated with rust from the susceptible first-generation hybrid $16(2\times 3)1$.

C.—On seedlings of partially resistant durum parent, Kubanka (CI 2094), inoculated with rust from the susceptible parent, Haynes Bluestem (Minnesota 169).

D.—On seedlings of partially resistant durum parent, Kubanka (CI 2094), inoculated with rust from the susceptible first-generation hybrid $16(2\times 3)1$.

PLATE 15

Puccinia graminis tritici

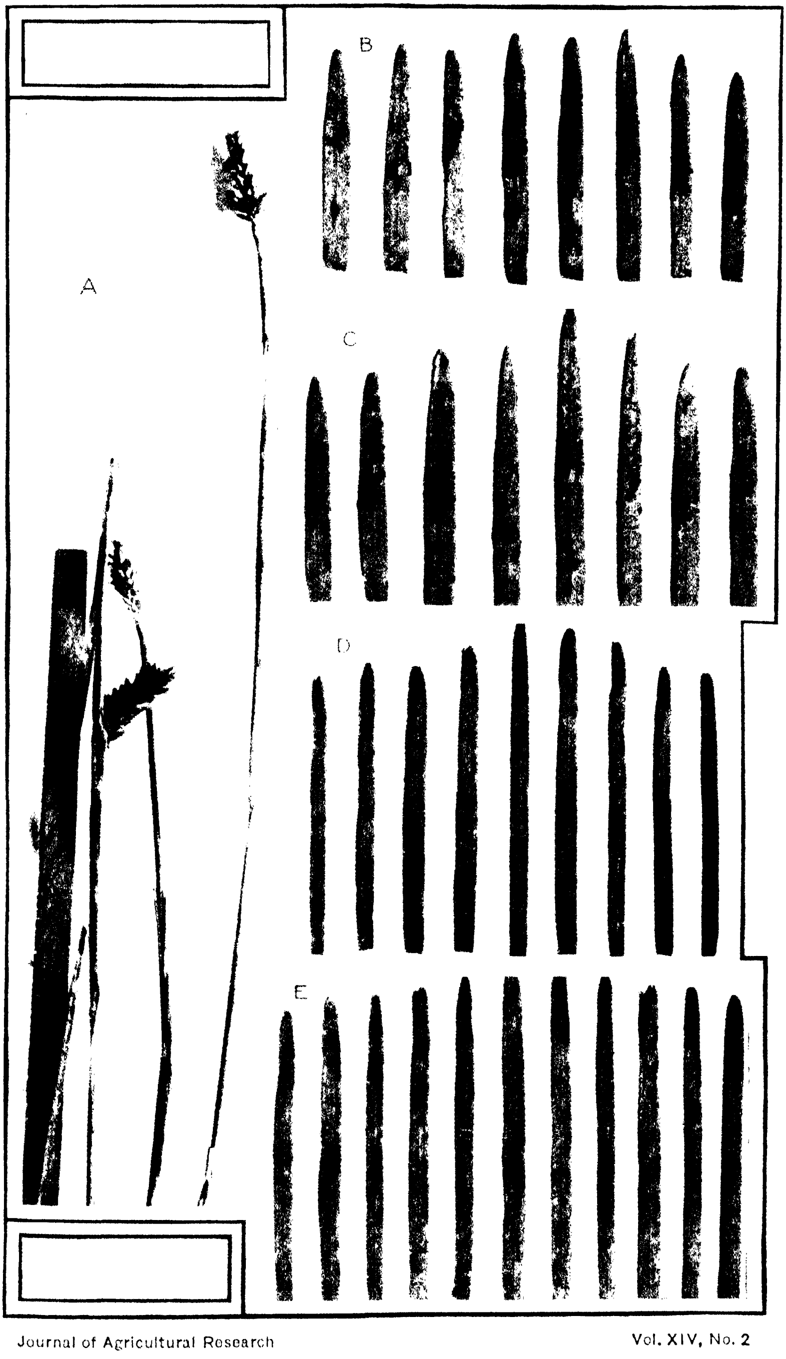
A.—Inoculations made on plants of susceptible second-generation hybrid of the cross emmer (Minnesota 1165) × Marquis.

B.—Seedlings of the susceptible parent, Marquis, inoculated with rust from the susceptible second-generation hybrid 15(8×1)1.

C.—Seedlings of the susceptible parent, Marquis, inoculated with rust from the stock cultures.

D.—Seedlings of extremely resistant parent, emmer (Minnesota 1165), inoculated with rust from the stock cultures.

E.—Seedlings of extremely resistant parent, emmer (Minnesota 1165), inoculated with rust from the susceptible second-generation hybrid 15(8×1)1.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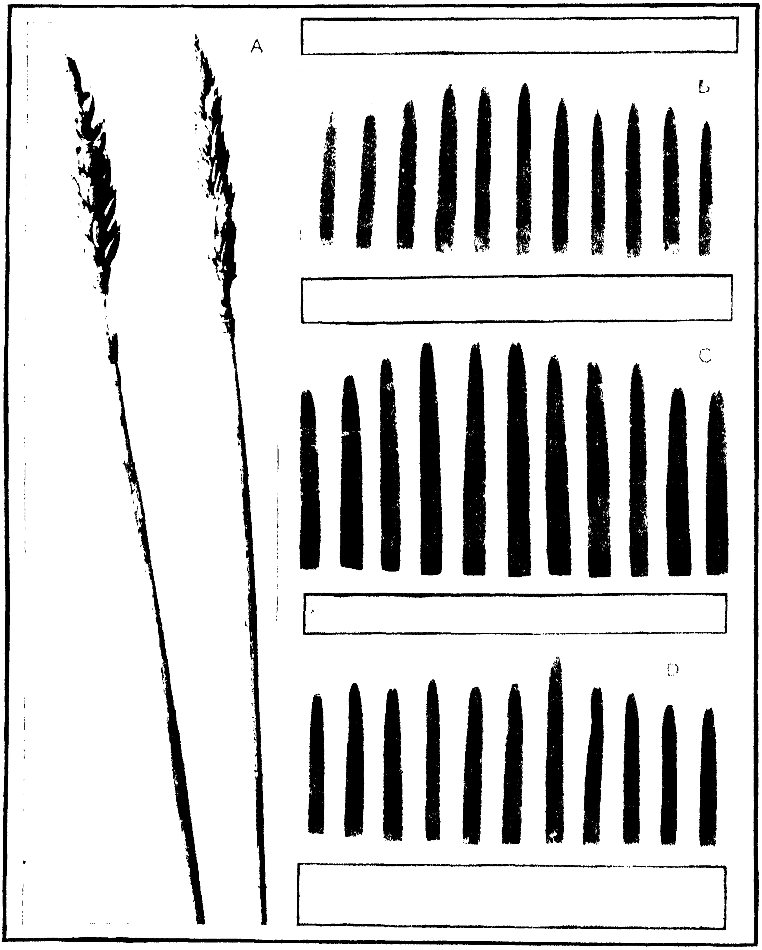
A
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PLATE 16

Puccinia graminis tritici

A.—Susceptible second-generation plants from the cross Marquis × Kubanka (CI 2094), inoculated with rust from the stock cultures.

B.—Seedlings of susceptible parent, Marquis, inoculated with rust from the susceptible second-generation plants shown in A.

C.—Seedlings of partially resistant parent, Kubanka (CI 2094), inoculated with rust from the stock cultures.

D.—Seedlings of partially resistant parent, Kubanka (CI 2094), inoculated with rust from susceptible second-generation plants shown in A.

PLATE 17

Puccinia graminis tritici

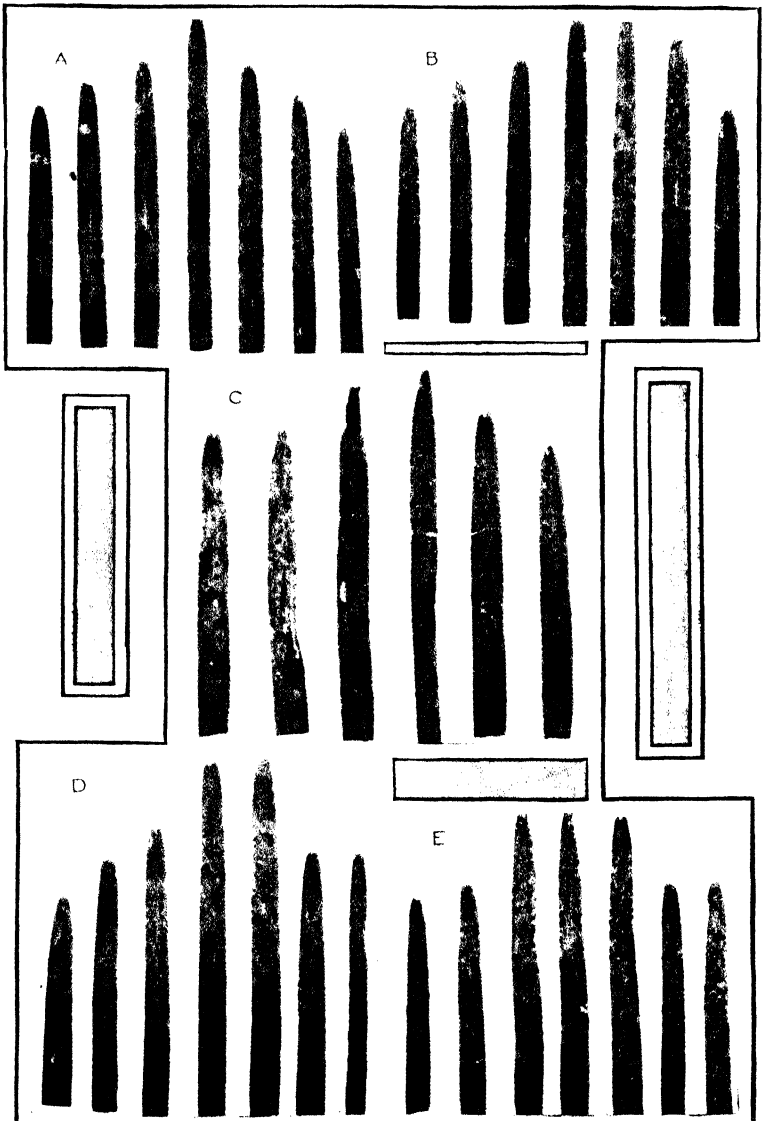
A.—Seedlings of susceptible parent, Haynes Bluestem (Minnesota 169), inoculated with rust from stock cultures.

B.—Seedlings of susceptible parent, Haynes Bluestem (Minnesota 169), inoculated with rust from the partially resistant F_9 hybrid (4×3)8AA shown in C.

C.—Seedlings of partially resistant F_9 hybrid (4×3)8 AA inoculated with rust from stock cultures.

D.—Seedlings of partially resistant parent, Kubanka (CI 2094), inoculated with rust from stock cultures.

E.—Seedlings of partially resistant parent, Kubanka (CI 2094), inoculated with rust from partially resistant F_9 hybrid (4×3)8AA shown in C.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MINERAL CONTENT OF SOUTHERN POULTRY FEEDS AND MINERAL REQUIREMENTS OF GROWING FOWLS

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INTRODUCTION

The mineral subtances which enter into the composition of fowls constitute the ash. These ash constituents stand in a peculiar and interesting relation to the living structures and life processes of animals. Through experimentation the physiologist has determined the effects of many of the minerals upon function and secretion. Minerals are also essential to the construction of the body tissues of the fowl. In this field the soil chemist, the agronomist, the poultryman, the physiologist, and the farmer all find a common interest, for the mineral substances required by fowls come from the soil through the plant to the bird. The force feeding of our fowls, both for growth into broilers and for egg production by mature hens, calls for a higher percentage of mineral nutrients in feedstuffs than was necessary under the old system of less intense production. Since it is so essential that fowls be supplied with sufficient amounts of each mineral element, studies should be conducted to determine whether any of our poultry feeds are deficient and, if so, how the deficiency may be remedied. It may not be possible to exercise a selective choice of feeds which provide an abundance of the various minerals required for rapidity or efficiency in the production of growth or eggs. The present paper deals only with experiments on minerals required in broiler production.

THE PROBLEM

Profitable broiler production begins with the baby chick and extends over a period of about eight weeks, at the end of which time the birds should weigh, as a flock, approximately 1½ pounds each.

Our problem consisted, therefore, in ascertaining (1) the amount of mineral per unit in the bodies of the baby chick and of the 1½-pound broiler; (2) the mineral content of the southern poultry feeds; (3) a proper feed mixture from the standpoint of protein, carbohydrate, and fat; (4) the mineral content of this mixture; and (5) by feeding, whether the

minerals of the feed mixtures were in sufficient quantities for the greatest rate of growth possible.

EXPERIMENTAL METHODS

The baby chicks were produced from a single flock of pure-bred Single-Comb White Leghorns bred at the Station and College poultry plant, and were hatched in one electric incubator, and each lot was housed under similar oil-burning hovers of 100-chick capacity. A room with a concrete floor and ample light and ventilation was used in which to rear the flocks. In this room the birds were confined to runs 6 feet square, having a smooth galvanized-iron floor laid over the concrete. A hover was set in the center of each run. The experiment was carried on in periods of seven days each, and extended over at least eight periods, or weeks. At the end of each period, after all material which adhered to the feet was carefully removed, the chicks were taken out and weighed, while the run was cleaned. The floor was swept and then scraped, care being taken not to scrape off any of the metal. It was then washed with distilled water, by the aid of a brush. This water was then drained into a pan, the material placed in an evaporating oven, and the moisture driven off. Though every care was taken to be accurate, there is a possibility that some errors may have crept in during these processes.

When fed in ordinary troughs, young chicks have a habit of throwing out their feed with their beaks, especially if they are not particularly hungry, and simply looking for something that might be very palatable to them. In order to avoid this wastage, we constructed the double boxes shown in Plate 18, *a*. Clabbered skim milk, the only liquid allowed, was given in 50-cc beakers, set in the ends of the boxes so that they could not be turned over. Chick-size limestone grit and chick-size oyster shell were given in petri dishes which were placed in another container (Pl. 18, *c*). Dry mash, a grain mixture, and cut green feed were placed in double boxes (Pl. 18, *b*, *d*, *e*). The feed thrown out of these double boxes was caught in the outside chamber, and was easily recovered. Rape was the green feed used (Pl. 18, *f*). The droppings which were deposited in the boxes were easily removed, after drying, by aid of a pair of forceps.

TABLE I.—*Mineral content of the bodies of the fowls* [a]

[Results expressed as parts per hundred]

Age.	Potassium.	Sodium.	Calcium.	Magnesium.	Sulphur.	Chlorin.	Phosphorus.	Iron.
Baby chick [b]	0.2922	0.2774	0.1978	0.0028	0.0107	0.1510	0.355	0.0054
½-pound broiler (Single-Comb White Leghorn)	.2380	.1580	1.0340	.0440	.3030	.0790	1.288	.0056
1-year-old hen (Columbian Wyandotte)	.2750	.1640	1.2970	.0510	.3820	.2080	1.510	.0066

[a] All chemical analyses in this work were made by Mr. Dan M. McCarty, Physiological Chemist, Animal Industry Division, North Carolina Experiment Station.

[b] The baby chicks were taken from the incubator, killed with chloroform, and their abdominal yolk sacs removed.

TABLE II.—*Mineral content of southern poultry feeds*

[Results expressed as parts per hundred]

SEPARATE ANALYSES

Feed.	Number of analyses.	Potassium.	Sodium.	Calcium.	Magnesium.	Sulphur.	Chlorin.	Phosphorus.	Iron.
Cornmeal, bolted	4	0.349	0.072	0.0092	0.1336	0.160	0.0244	0.341	0.004
Pinhead oats	7	.441	.109	.0126	.0704	.236	.0900	.499	.0019
Rolled oats	2	.370	.136	.0430	.1560	.256	.0238	.473	.0062
Whole wheat	13	.435	.039	.0271	.1127	.183	.0630	.436	.007
Whole corn	13	.332	.041	.0127	.1051	.148	.0521	.293	.0044
Wheat middlings	6	.949	1.219	.0980	.3628	.232	.0603	.783	.0052
Bone meal [a]	3	.229	.735	21.1770	.5800	.170	.0900	10.349	.018
Hulled oats	6	.387	.053	.0915	.1465	.204	.0870	.454	.0109
Meat and bone meal [a]	4	.185	.745	12.806	.460	.359	.850	6.560	.0580
Velvet-bean meal	1	1.186	.141	.360	.208	.151	.222	.764	.0126
Soy bean meal (fat extract)	1	1.189	.415	.238	.298	.438	.032	.664	.0300
Peanut meal (fat extract)	1	1.177	.326	.138	.326	.323	.043	.735	.0300
Skim milk	2	.151	.144	.153	.0018	.0424	.065	.136	.0036
Egg, including shell	3	.0103	.200	.608	.0985	.3950	.150	.302	.0103
Rape, green	3	.2510	.008	.0084	.0206	.0354	.093	.1026	.00000076
Limestone grit	2	.0000	.000	30.9700	6.6700	.000	.000	.000	3.330000
Oyster shell	2	.0000	.000	37.951	.4200	.147	.090	.000	.375000

ANALYSES OF FEED IN EACH SACK [b]

Feed.		Potassium.	Sodium.	Calcium.	Magnesium.	Sulphur.	Chlorin.	Phosphorus.	Iron.
(1a) Corn meal, bolted		0.265	0.076	0.0102	0.1318	0.103	0.0232	0.3310	0.0018
(1b) Pinhead oats		.407	.115	.0100	.0557	.264	.1050	.5190	.0020
(1c) Rolled oats		.370	.140	.0430	.1557	.276	.0249	.4020	.0072
(4d) Cracked wheat		.437	.044	.0240	.1027	.182	.0650	.4240	.0022
(1e) Cracked corn		.344	.038	.0170	.0667	.195	.0550	.3012	.0028
(1f) Wheat middlings		.694	.646	.0840	.3728	.236	.1050	.9520	.0048
(1g) Bone meal		.145	.886	22.0550	.5700	.180	.0700	10.8600	.0150
(1h) Meat and bone meal		.179	.304	13.[illegible]	.2700	.350	.8200	6.5700	.0500
(1i) Hulled oats		.374	.084	.076	.1542	.235	.0600	.4260	.0030
(1j) Limestone grit		.000	.000	30.970	6.0700	.000	.0000	.0000	3.3300
(1k) Oyster shell		.000	.000	37.951	.4200	.147	.0080	.0000	.3750
(1m) Skim milk		.151	.144	.153	.0018	.0424	.0650	.1360	.0036

[a] It is probable that in both bone meal and in meat and bone meal considerable tricalcium phosphate [$Ca_3(PO_4)_2$] is lost in burning.

[b] In making up the feed mixtures for these experiments each kind of feed was taken from a single sack.

FEED MIXTURES

The following mixtures were used in these experiments, the proportions being given by weight:

MIXTURE 1

1c Rolled oats	8 parts.
1f Wheat middlings	8 parts.
1h Meat and bone meal	2 parts.
1g Bone meal	1 part.

MIXTURE 2

4d Cracked wheat	3 parts.
1e Cracked corn	2 parts.
1b Pinhead oats	1 part.

MIXTURE 3

1f Wheat middlings	6 parts.
1a Corn meal	3 parts.
1h Meat and bone meal	3 parts.
1g Bone meal	1 part.

MIXTURE 4

4d Whole wheat	3 parts.
1e Cracked corn	2 parts.
1i Hulled oats	1 part.

Table III gives the mineral content of the four feed mixtures.

TABLE III.—*Mineral content of feed mixtures*

[Results expressed as parts per hundred]

Mixture.	Potassium.	Sodium.	Calcium.	Magnesium.	Sulphur.	Chlorin.	Phosphorus.	Iron.
1	0.4742	0.4133	2.61764	0.2809	0.2623	0.1447	1.8281	0.0110
2	.409	.048	.01920	.0820	.200	.0650	.4289	.0023
3	.433	.453	4.81446	.3080	.242	.2482	2.8248	.0153
4	.394	.043	.02699	.0992	.195	.0608	.4100	.0020

TABLE IV.—*Feed and mineral intake of lot 3*

Period and feed.	Quantity of feed consumed.	Potassium.	Sodium.	Calcium.	Magnesium.	Sulphur.	Chlorin.	Phosphorus.	Iron.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
FIRST PERIOD:									
Milk	1,510	2.280	2.174	2.310	0.0271	0.6402	0.9815	2.0536	0.0543
Mixture 1	299	1.417	1.235	7.826	.8398	.7842	.4326	5.4660	.0328
Mixture 2	45	.184	.027	.008	.0369	.0900	.0292	.1930	.0010
Grit	76	.000	.000	23.537	5.0692	.0000	.0000	.0000	2.5308
Total	1,930	3.881	3.436	33.678	5.9730	1.5144	1.4433	7.7126	2.6189
SECOND PERIOD:									
Milk	2,492	3.762	3.588	3.812	0.0448	1.0566	1.6198	3.3801	0.0897
Mixture 1	391	1.854	1.616	10.234	1.0933	1.0255	.5657	7.1478	.0430
Mixture 2	275	1.124	.132	.052	.2255	.5500	.1787	1.1794	.0063
Rape	156	.391	.012	.013	.0321	.0552	.1450	.1600	Trace.[a]
Total	3,314	7.131	5.348	14.111	1.4007	2.6873	2.5092	11.8763	.1390
THIRD PERIOD:									
Milk	2,356	3.557	3.392	3.604	0.0424	0.9989	1.5314	3.2041	0.0848
Mixture 1	499	2.366	2.062	13.062	1.4016	1.3088	.7220	9.1222	.0099
Mixture 2	368	1.505	.176	.070	.3017	.7360	.2392	1.5783	.0084
Rape	385	.966	.030	.032	.0793	.1362	.3580	.3930	Trace.
Grit	22	.000	.000	6.813	1.4674	.0000	.0000	.0000	.7326
Oyster shell	11	.000	.000	4.174	.0462	.0161	.0099	.0000	.0412
Total	3,641	8.394	5.660	27.755	3.3386	3.1960	2.8005	14.2096	.8769
FOURTH PERIOD:									
Milk	2,545	3.842	3.664	3.893	0.0458	1.0790	1.6542	3.4612	0.0916
Mixture 2	547	2.237	.262	.105	.4485	1.0940	.3555	2.3400	.0125
Mixture 3	523	2.264	2.369	25.179	1.6108	1.2656	1.2980	14.7737	.0800
Rape	342	.858	.027	.028	.0704	.1210	.3180	.5308	Trace.
Grit	8	.000	.000	2.477	.5336	.0000	.0000	.0000	.2664
Oyster shell	17	.000	.000	6.451	.0714	.0249	.0153	.0000	.0637
Total	3,982	9.201	6.322	38.133	2.7805	3.5845	3.6410	21.1117	.5142
FIFTH PERIOD:									
Milk	3,248	4.904	4.677	4.969	0.0584	1.3771	2.1112	4.4172	0.1196
Mixture 2	1,247	5.100	.598	.239	1.0225	2.4940	.8105	5.3483	.0286
Mixture 3	334	1.446	1.513	16.080	1.0287	.8082	.8289	9.4348	.0511
Rape	321	.805	.025	.026	.0661	.1136	.2985	.3293	Trace.
Grit	18	.000	.000	5.574	1.2006	.0000	.0000	.000	.5994
Total	5,168	12.255	6.813	26.888	3.3763	4.7929	4.0491	19.5296	.7960
SIXTH PERIOD:									
Milk	3,400	5.134	4.896	5.202	0.0612	1.4416	2.2100	4.6240	0.1224
Mixture 2	1,353	5.533	.649	.259	1.1094	2.7060	.8794	5.8030	.0311
Mixture 3	381	1.649	1.725	18.343	1.1734	.9220	.9456	10.7624	.0582
Rape	912	2.289	.072	.076	.1878	.3228	.8481	.9357	Trace.
Grit	33	.000	.000	10.220	2.2011	.0000	.0000	.0000	1.0989
Total	6,079	14.605	7.342	34.100	4.7329	5.3924	4.8831	22.1251	1.3106

[a] These estimates are made on the basis of the green plant as stated in an early part of this paper, and hence the intake is small.

TABLE IV—*Feed and mineral intake of lot 3*—Continued

Period and feed.	Quantity of feed consumed.	Potassium.	Sodium.	Calcium.	Magnesium.	Sulphur.	Chlorin.	Phosphorus.	Iron.
SEVENTH PERIOD:	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Milk	3,989	6.023	5.744	6.103	0.0718	1.6913	2.5928	5.4250	0.1436
Mixture 3	332	1.437	1.503	15.984	1.0225	.8034	.8240	9.3783	.0507
Mixture 4	1,236	4.869	.531	.333	1.2261	2.4102	.7514	5.0676	.0247
Rape	880	2.208	.070	.073	.1812	.3115	.8184	.9028	Trace.
Grit	39	.000	.000	12.078	2.6013	.0000	.0000	.0000	1.2987
Total	6,476	14.537	7,848	34.571	5.1029	5.2164	4.9866	20.7737	1.5177
EIGHTH PERIOD:									
Milk	4,203	6.346	6.052	6.430	0.0756	1.7820	2.7319	5.7160	0.1513
Mixture 3	382	1.654	1.730	18.391	1.1765	.9244	.9481	10.7907	.0584
Mixture 4	1,484	5.846	.638	.400	1.4721	2.8938	.9022	6.0844	.0296
Rape	731	1.834	.058	.061	.1505	.2587	.6798	.7500	Trace.
Total	6,800	15.680	8.478	25.282	2.8747	5.8589	5.2620	23.3411	.2393

TABLE V.—*Weights of the chicks at hatching and at the end of each of the eight periods*

Chick No.	Weight at—								
	Hatching.	7 days.	14 days.	21 days.	28 days.	35 days.	42 days.	49 days.	56 days
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
902	41	57	91	128	135	205	280	350	460
904	45	57	83	123	165	215	253	265	316
906	41	59	88	132	169	245	311	305	430
910	40	60	98	145	197	245	308	355	420
912	42	58	85	110	168	219	287	335	411
914	40	52	65	113	149	205	278	320	400
918	42	59	75	110	151	203	252	275	329
921	42	70	77	108	154	200	271	335	438
922	36	57	90	137	187	250	300	345	410
924	43	59	90	130	150	185	215	240	282
926	44	57	[a] 79	......	......	......	......	......	......
928	42	45	[a] 65	......	......	......	......	......	......
Total	498	690	986	1,236	1,625	2,172	2,755	3,185	3,896

[a] Died.

TABLE VI.—*Percentage increase in weight at the end of each 7-day period*

Chick No.	Period No.							
	1	2	3	4	5	6	7	8
902	28	37	28	5	34	26	20	23
904	21	31	32	25	23	15	4	16
906	30	31	33	21	31	21	14	15
910	50	38	32	26	15	20	13	15
912	27	31	22	28	23	24	14	16
914	23	20	42	24	27	26	13	13
918	28	20	31	20	25	19	8	16
921	40	10	28	29	23	20	19	23
922	36	36	34	26	25	16	13	15
924	27	34	30	13	18	13	10	14
926	22	[a] 27	......	......	......	......	......	......
928	6	[a] 30	......	......	......	......	......	......
Flock average	27	30	31	23	25	21	13	18
Total gain ... gms.	192	296	394	389	547	583	430	711

[a] Died.

TABLE VII.—*Amount of gain in each period and the amount of mineral elements required to build up this amount of tissue, based on the analyses of the bodies of the baby chick and the 1½-pound broiler*

BABY CHICKS

Period.	Gain.	Potassium.	Sodium.	Calcium.	Magnesium.	Sulphur.	Chlorin.	Phosphorus.	Iron.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
First	192	0.5010	0.5326	0.3797	0.0053	0.0205	0.2899	0.6816	0.0103
Second	296	.8649	.8211	.5854	.0082	.0310	.4469	1.0508	.0159
Third	394	1.1512	1.0929	.7703	.0110	.0421	.5949	1.3987	.0212
Fourth	389	1.1366	1.0790	.7604	.0108	.0416	.5873	1.3809	.0210
Fifth	547	1.5983	1.5173	1.0819	.0153	.0585	.8259	1.9418	.0295
Sixth	583	1.7035	1.6172	1.1531	.0163	.0623	.8803	2.0696	.0314
Seventh	430	1.2564	1.1928	.8505	.0120	.0460	.6493	1.5265	.0232
Eighth	711	2.0775	1.9723	1.4063	.0199	.0760	1.0736	2.5240	.0383

1½-POUND SINGLE-COMB WHITE LEGHORN BROILERS

Period.	Gain.	Potassium.	Sodium.	Calcium.	Magnesium.	Sulphur.	Chlorin.	Phosphorus.	Iron.
First	192	0.4569	0.3033	1.9852	0.0844	0.5817	0.1516	2.4729	0.0107
Second	296	.7044	.4676	3.0606	.1302	.8968	.2338	3.8124	.0165
Third	394	.9377	.6225	4.0739	.1733	1.1938	.3112	5.0747	.0220
Fourth	389	.9258	.6146	4.0222	.1711	1.1786	.3073	5.0103	.0217
Fifth	547	1.3018	.8642	5.6559	.2406	1.6574	.4321	7.0453	.0306
Sixth	583	1.3875	.9211	6.0282	.2565	1.7664	.4605	7.5090	.0326
Seventh	430	1.0234	.6794	4.4462	.1892	1.3029	.3397	5.5384	.0240
Eighth	711	1.6921	1.1233	7.3517	.3128	2.1543	.5016	9.1576	.0398

TABLE IX.—*Mineral intake supplied by the feed, the outgo by way of the bowel, the amount of each element required to build up the tissue gain, based on the analyses of the bodies of 1½-pound broilers, and the mineral balance*

Period and factor.	Potassium.	Sodium.	Calcium.	Magnesium.	Sulphur.	Chlorin.	Phosphorus.	Iron.
First period:	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Intake	3.8810	3.4360	33.0780	5.9730	1.5144	1.4433	7.7126	2.6189
Outgo	1.9847	1.0445	16.7895	2.3497	.6728	.7905	3.8349	.0020
Required	.4569	.3033	1.9852	.0844	.5817	.1516	2.4729	.0107
Balance	+ 1.4394	+ 2.0882	+ 14.9233	+ 3.5389	+ .2599	+ .5012	+ 1.4048	+ 2.6062
Second period:								
Intake	7.1310	5.3480	14.1110	1.4007	2.6873	2.5092	11.8763	0.1390
Outgo	5.7272	2.2206	15.9993	5.1619	2.3389	2.0876	8.3507	.0203
Required	.7044	.4676	3.0606	.1302	.8968	.2338	3.8124	.0165
Balance	+ .6994	+ 2.6598	− 4.9489	− 3.8914	− .5484	+ .1878	− .2868	+ .1022
Third period:								
Intake	8.3940	5.6600	27.7550	3.3386	3.1960	2.8605	14.2996	0.8769
Outgo	6.4260	3.0704	17.0000	2.4208	1.3804	3.1790	9.1120	.0340
Required	.9377	.6225	4.0739	.1733	1.1938	.3112	5.0747	.0220
Balance	+ 1.0303	+ 1.9671	+ 6.6811	+ .7445	+ .6218	− .6297	+ .1129	+ .8209
Fourth period:								
Intake	9.2010	6.3220	38.1330	2.7805	3.5845	3.6410	21.1110	0.5142
Outgo	7.7360	4.5841	30.4734	2.6014	1.8724	2.8585	16.9410	.0314
Required	.9258	.6146	4.0222	.1711	1.1786	.3073	5.0105	.0217
Balance	+ .5392	+ 1.1233	+ 3.6374	+ .0080	+ .5335	+ .4752	− .8405	+ .4611
Fifth period:								
Intake	12.2550	6.8130	26.8880	3.3763	4.7929	4.0491	19.5296	0.7960
Outgo	9.5486	3.7437	32.1895	3.6274	2.6184	3.4894	14.9857	.0324
Required	1.3018	.8642	5.6559	.2406	1.6574	.4321	7.0453	.0306
Balance	+ 1.4046	+ 2.2051	+ 10.9574	− .4917	+ .5171	+ .1276	− 2.5014	+ .7330

TABLE IX.—*Mineral intake supplied by the feed, the outgo by way of the bowels, the amount of each element required to build up the tissue gain, based on the analyses of the bodies of 1½-pound broilers, and the mineral balance*—Continued

Period and factor.	Potassium.	Sodium.	Calcium.	Magnesium.	Sulphur.	Chlorin.	Phosphorous.	Iron.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Sixth period:								
Intake	14.6050	7.3420	34.1000	4.7329	5.3924	4.8831	22.1251	1.3106
Outgo	13.1084	5.9358	31.8477	5.0382	3.0062	3.4293	18.0121	.0448
Required	1.3875	.9211	6.0282	.2565	1.7664	.4605	7.5090	.0326
Balance	+ .1091	+ .4851	− 3.7759	− .5618	+ .6198	+ .9933	− 3.3960	+1.2332
Seventh period:								
Intake	14.5370	7.8480	34.5710	5.1029	5.2164	4.9866	20.7737	1.5177
Outgo	13.3769	4.4705	21.7799	3.3716	3.3460	3.8460	16.6019	.0233
Required	1.0234	.6794	4.4462	.1892	1.3029	.3397	5.5384	.0240
Balance	+ .1367	+ 2.6981	+ 8.3949	+1.5421	+ .5675	+ .8009	− 1.3666	+1.4704
Eighth period:								
Intake	15.6800	8.4780	25.2820	2.8747	5.8589	5.2620	23.3411	0.2393
Outgo	16.5110	5.9803	33.2590	5.9082	4.0527	4.5030	19.2760	.0395
Required	1.6921	1.1233	7.3517	.3128	2.1543	.5616	9.1576	.0398
Balance	− 2.5231	+ 1.3744	−15.3287	−3.4263	− .3481	+ .1974	− 5.1576	+ .1600
Total balance	+ 2.8356	+14.6011	+20.5400	−2.5371	+2.2231	+2.6542	−12.0312	+7.5870

TABLE VIII.—*Total weight of mineral in the droppings for each period*

Period.	Potassium.	Sodium.	Calcium.	Magnesium.	Iron.	Phosphorus.	Sulphur.	Chlorin.	Total droppings.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
First	1.9847	1.0445	16.7695	2.3497	0.0020	3.8340	0.6728	0.7905	168.2
Second	5.7272	2.2206	15.9993	5.1619	.0203	8.3507	2.3389	2.0876	309.5
Third	6.4260	3.0704	17.0000	2.4208	.0340	9.1120	1.3804	3.1790	340
Fourth	7.7363	4.5841	30.4734	2.6014	.0314	16.9410	1.8724	2.8585	524.5
Fifth	9.5486	3.7437	32.1895	3.6274	.0324	14.9857	2.6184	3.4894	541
Sixth	13.1084	5.9358	31.8477	5.0382	.0448	18.0121	3.0062	3.4293	641
Seventh	13.3769	4.4705	21.7299	3.3716	.0233	16.6019	3.3460	3.8460	641
Eighth	16.5110	5.9803	33.2590	5.9882	.0395	19.2760	4.0527	4.5030	790

DISCUSSION OF RESULTS

In studying the mineral content of poultry feeds we found that the different samples of products grown in different sections of the country, or even different sections of a State, or on different plots of ground, vary in their inorganic content. Therefore we have given the averages of a large number of analysis from different lots of the same kind of feeds in order to show the average of these specific analyses (Table II) and later a tabulation of the analyses of just the feeds used in these experiments (Table III). The latter table will enable us to determine definitely just the amount of mineral taken in in these feeding experiments. Not only the poverty of the soil, but also seasonal variations from year to year, such as drouth, may affect the mineral content of the feed.

In the baby chick the bones are very thin walled and bend easily, indicating that thorough calcification has not taken place in all parts. It is to be expected that later in the life of the chick there would be a greater amount of mineral in the bones and consequently a greater percentage in the total weight of the bird. If the results of the analyses

which were carried on with the bodies of baby chicks and of 1½-pound broilers be studied, it will be noted that there is a material increase of the greater essential inorganic constituents of the bone—namely, calcium, magnesium, and phosphorus. The baby chick is provided with down. This is gradually replaced with a coat of feathers as the chick develops, which calls for an increase of sulphur. It will also be noted that in this element there has been a material increase. To carry our comparative study a step farther, it will be noted that, as the bird develops to maturity, there is a still greater increase in calcium, magnesium, phosphorus, and sulphur, though the iron content is only slightly increased.

The bird has no sweat glands and only one oil gland, the latter a double lobulated tubular gland located dorsally at the base of the tail. This gland furnishes oil for the bird to distribute to each feather by the aid of its beak. The excretions of the body of the fowl are cast off by way of the lungs, kidneys, and intestinal tract. The ureters, large intestine, oviduct, and vasa deferentia all empty into a reservoir, an expansion of the terminal end of the gut, called the "cloaca," which in turn empties through the anus to the external world. This arrangement makes the isolation of elements eliminated by the kidneys a difficult task; in fact, it is impossible except through surgical interference, and this has many difficulties. In these particular experiments we have not attempted this.

In an average of two lots in this series of experiments the following quantities of feeds were required to produce 1 gm. of gain in weight: Milk, 7.49 gm.; mash and grain mixtures, 2.91 gm.; green feed, 1 gm.; total 11.44 gm. In these cases the feed was kept constantly before the flocks, so that the consumption was a maximum amount and by selection, so far as the milk, mash, and grain mixtures were concerned. The rape, finely chopped, and the milk were likewise kept in separate containers. Thus, where the chicks in their first eight weeks are given all the sour skim milk and green feed they will consume, there will be required approximately 3 gm. of grain and mash per gram of gain in body weight. In these two lots it was found that 75.2 per cent of the carbohydrates were digested and 80.2 per cent of the fat. These are the averages for the eight periods, the digestibility varying from period to period.

The methods used to separate the ammonia and uric acid of the feces from the undigested protein of the feed were not considered to be sufficiently accurate to give here. The problem of separating the mineral elements from those passed out with the feces unused is a quite different matter. If it were possible to separate the urine from the feces by surgical interference, there would yet remain that eliminated by way of the bowel, which could not be separated from that taken with the food and not utilized. At this time there seems to be only one practical way to measure mineral requirements—that is, by comparing the intake with the outgo and the amount required to construct the tissue gain, and to study the mineral balances left over unaccounted for.

From Table I, which gives the mineral content of the bodies of fowls, may be seen the requirement in utilizable mineral needed to construct a given gain. From the above estimate of the quantity of feeds to produce a pound of gain can be estimated the amount of mineral elements contained in the feed.

The mineral intake will fluctuate with the kinds of materials given in addition to the dry mash and the grain mixtures. By a study of the table of average analyses (Table II) it will be seen that milk contains quite a large quantity of phosphorus, calcium, sodium, and potassium, and hence the intake of sour skim milk, if the chicks are given all they will consume, supplies much in the way of mineral elements. Thus, in the first period 58 per cent of the potassium, 63 per cent of the sodium, 6 per cent of the calcium[1] 0.0045 per cent of magnesium,[1] 42 per cent of sulphur, 68 per cent of chlorin, 26 per cent phosphorus, and 2 per cent of iron[1] were furnished by the sour skim milk.

In the second period 52 per cent of the potassium, 67 per cent of the sodium, 27 per cent of the calcium, 3 per cent of the magnesium, 39 per cent of the sulphur, 64 per cent of the chlorin, 28 per cent of the phosphorus, and 64 per cent of the iron were furnished by the sour skim milk.

In the third period 42 per cent of the potassium, 60 per cent of the sodium, 13 per cent of the calcium, 1 per cent of the magnesium, 31 per cent of the sulphur, 53 per cent of the chlorin, 22 per cent of the phosphorus, and 9 per cent of the iron were furnished by the milk.

In the fourth period 41 per cent of the potassium, 57 per cent of the sodium, 10 per cent of the calcium, 1 per cent of the magnesium, 30 per cent of the sulphur, 45 per cent of the chlorin, 16 per cent of the phosphorus, and 17 per cent of the iron were furnished by the sour skim milk.

In the fifth period 40 per cent of the potassium, 68 per cent of the sodium, 18 per cent of the calcium, 1 per cent of the magnesium, 28 per cent of the sulphur, 52 per cent of the chlorin, 22 per cent of the phosphorus, and 14 per cent of the iron were furnished by the sour skim milk.

In the sixth period 35 per cent of the potassium, 66 per cent of the sodium, 15 per cent of the calcium, 1 per cent of the magnesium, 26 per cent of the sulphur, 45 per cent of the chlorin, 20 per cent of the phosphorus, and 9 per cent of the iron were furnished by the sour skim milk.

In the seventh period 41 per cent of the potassium, 73 per cent of the sodium, 17 per cent of the calcium, 1 per cent of the magnesium, 32 per cent of the sulphur, 52 per cent of the chlorin, 26 per cent of the phosphorus, and 9 per cent of the iron were supplied by the sour skim milk.

[1] It must be remembered that all the mineral elements in the grit are not liberated for use in the same period in which it is consumed, since all the grit will not be ground for about two weeks.

In the eighth period 40 per cent of the potassium, 70 per cent of the sodium, 25 per cent of the calcium, 2 per cent of the magnesium, 32 per cent of the sulphur, 51 per cent of the chlorin, 24 per cent of the phosphorus, and 63 per cent of the iron were supplied by the sour skim milk.

If we consider the total mineral nutrients that would have been supplied by the feed ingested, leaving out the milk, there would have been ample furnished in any of the eight periods.

If the birds had received neither milk nor mash, there would have been a deficiency in the first period in all mineral elements except potassium and magnesium provided that the same quantity of feed was consumed as a grain mixture and that the consumption of shell or limestone as grit was not considered. In the second period there would have been a deficiency in sodium, calcium, potassium, and iron. In the third period there would have been a deficiency in sodium, calcium, phosphorus, and iron. In the fourth period there would have been the same deficiency as in the third period. In the fifth, sixth, and seventh periods there would have been a deficiency in calcium alone, and a deficiency in calcium, sodium, and phosphorus in the eighth period.

In Table IX there is an apparent balance of calcium of 20 gm. unaccounted for, and in this connection it must be remembered there would be at least 1 or 2 gm. of limestone grit per bird still remaining in the gizzards at the end of these tests. This would likewise affect the magnesium, leaving a small balance, and the same holds good for the iron, since the limestone used in these experiments contained 3 per cent of iron. The summary of the eight periods indicates an apparent shortage of phosphorus and a slight shortage of magnesium.

To supply the proper amount of phosphorus, magnesium, and calcium to growing chicks, in mashes consisting of such mill feeds as middlings and ground oats there should be added meat and bone meal, or bone meal, or meat meal. Sour skim milk and buttermilk, if given in sufficient quantities, aid in making good the mineral shortages as well as providing food hormones, which have a stimulating effect upon the growth of the young, as shown by work in this and other laboratories.

SUMMARY

The mineral content of southern poultry feeds varies in different kinds of feed and in different lots of the same kind. This difference is influenced by weather conditions, such as drouth, and by the different mineral contents of the soil.

In the development of the broiler from the baby chick there is a gradual increase in the requirements of calcium, magnesium, phosphorus, and sulphur. To supply this increase and to attain the best growth there must be added to a ration consisting of mill products and ground grain, such products as meat meal, bone meal, meat and bone meal, and sour skim milk or buttermilk.

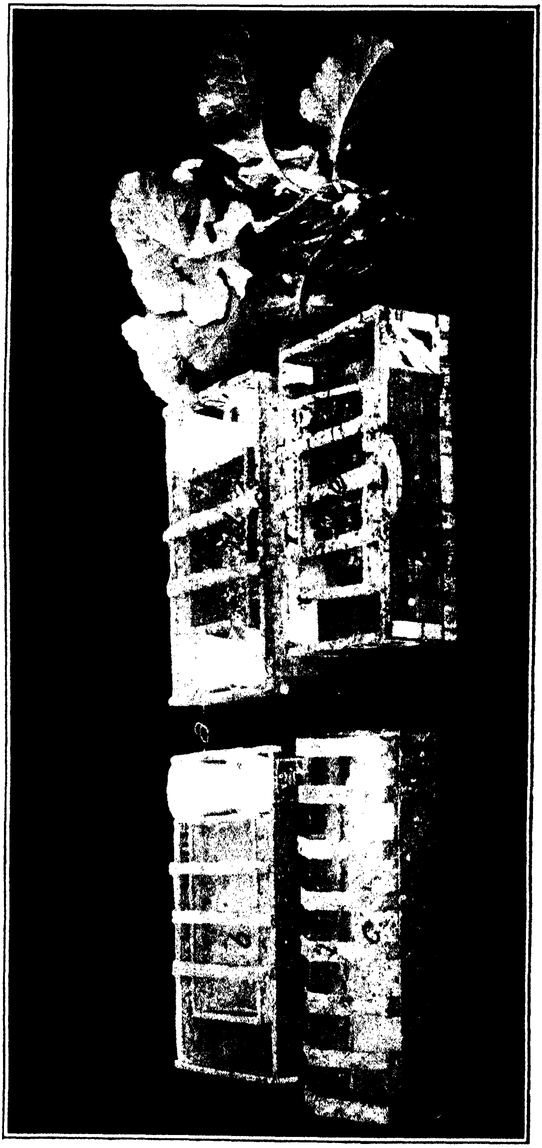

PLATE 18

Feeding utensils used in the feeding experiments with chicks: *a*, Beakers for the sour skim milk; *b*, container for the dry mash; *c*, container for grit or shell; *d*, container for the grain mixture; *e*, container for the green feed; *f*, rape used as green feed.

FEMALE LEPIDOPTERA AT LIGHT TRAPS

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INTRODUCTION

It appears to be the generally accepted theory that in the Lepidoptera practically all individuals taken at a light trap are males, and that of the few females so captured all have oviposited previously. During the summer of 1916 extended observations were made at the Hagerstown, Maryland, field station of the Bureau of Entomology in an effort to secure some definite information as to the relative proportions of the sexes of moths attracted to the light and the percentage of gravid females among those so taken. The purpose of this paper is to give a brief account of the methods employed to obtain material and a summary of the facts brought out by a detailed examination of such material.

The attracting light used was an arc lamp of 300 candlepower hung in an inverted truncated cone of heavy tin. One-half of the cone which would otherwise encircle the lamp was cut away; the narrow (lower) end of the cone was fitted in the circular opening in the top of the trap. Immediately below this opening are arranged several plates of glass at angles to direct the moths downward into the body of the trap. The trap is 12 by 14 inches and 20 inches high. Two sides are of wire mesh, the other sides and the top and bottom being of wood. To kill the captured insects, the trap was placed in a tightly constructed box with a small vessel of carbon disulphid placed at the top of the trap.

The individuals of some twenty-odd species were preserved in alcohol, with the date of each collection. Later these were determined as to sex and the number of males and females tabulated for each date. The females were carefully dissected and tabulated as to the stage of ova development.

[1] The writer wishes to express his appreciation of the assistance given by Mr. Harry L. Parker, of the Hagerstown station, who separated the individuals of Caenurgia into the two species represented; and to acknowledge the help received from other men of the station.

No attempt was made to determine specifically the individuals of the genus Feltia, of which it is probable that the following four species were taken: *Feltia subgothica* Haworth, *F. annexa* Treitschke, *F. gladiaria* Morrison, and *F. jaculifera* Guenée.

The material collected and examined embraces a little over 11,000 individuals, representing 3 families and about 20 species. Table I gives the results of an examination of this large number of moths to determine the sex. No extended résumé is attempted in the text beyond a brief statement of some of the more salient facts.

Of the 11,222 moths examined, 8,025, or 71.5 per cent, were males; 3,197, or 28.5 per cent, were females. In only one species, *Noctua c-nigrum,* did the females taken equal or exceed the males.

TABLE I.—*Number and percentage of males and females of various species of Lepidoptera taken at a light trap, Hagerstown, Md., 1916*

Species.	Number of males.	Number of females.	Total.	Percentage of males.	Percentage of females.
Apantesis vittata Fabricius	1,158	25	1,183	97.9	2.1
Apantesis arge Drury	14	3	17	82.3	17.7
Estigmene acraea Drury	404	69	473	85.4	14.6
Diacrisia virginica Fabricius	66	8	74	88.0	12.0
Isia isabella Smith and Abbot	256	42	298	86.0	14.0
Halisidota tessellaris Smith and Abbot	282	123	405	69.6	30.4
Datana ministra Drury	47	19	66	71.2	28.8
Arsilonche albovenosa Goeze	111	11	122	90.0	10.0
Autographa biloba Stephens	38	2	40	95.0	5.0
Autographa simplex Guenée	223	71	294	75.8	24.2
Meliana diffusa Walker	159	19	178	89.4	10.6
Polia renigera Stephens	192	77	269	71.4	28.6
Caenurgia erechtea Cramer	1,437	833	2,270	64.1	35.9
Caenurgia crassiuscula Haworth	973	566	1,539	64.5	35.5
Cirphis unipuncta Haworth	552	424	976	56.5	43.5
Noctua c-nigrum Linnaeus	95	107	202	47.0	53.0
Feltia spp	2,018	798	2,816	71.7	28.3
Total	8,025	3,197	11,222	71.5	28.5

Table II gives the percentage of gravid females and shows that of 3,197 individuals dissected, 1,857, or 58 per cent, were gravid. These gravid females make up 16.6 per cent of the 11,222 moths examined.

It will be noted that all the females of four of the six species of Arctiidae under observation were gravid, and in the two other species the gravid females represent 85.5 per cent and 96 per cent of females collected. These facts, together with data as to the number and development of the eggs, are to be found in Table III.

TABLE II.—*Number and percentage of gravid female Lepidoptera taken at a light trap, Hagerstown, Md., 1916*

Species.	Number of females taken.	Spent.	Gravid.	
			Number.	Per cent.
Apantesis vittata Fabricius	25	0	25	100.0
Apantesis arge Drury	3	0	3	100.0
Estigmene acraea Drury	69	10	69	85.5
Diacrisia virginica Fabricius	8	0	8	100.0
Isia isabella Smith and Abbot	42	0	42	100.0
Halisidota tessellaris Smith and Abbot	123	5	118	96.0
Autographa biloba Stephens	2	2	0	0.0
Autographa simplex Guenée	71	19	52	73.0
Meliana diffusa Walker	19	12	7	37.0
Polia renigera Stephens	77	10	67	87.0
Caenurgia erechtea Cramer	833	389	444	53.3
Caenurgia crassiuscula Haworth	566	290	276	48.7
Cirphis unipuncta Haworth	424	85	339	80.0
Noctua c-nigrum Linnaeus	107	58	49	54.8
Arsilonche albovenosa Goeze	11	0	11	100.0
Feltia spp	798	394	404	50.6
Datana ministra Drury	19	0	19	100.0
Total	3,197	1,340	1,857	58.0

TABLE III.—*Condition of the ovaries of Lepidoptera taken at a light trap, Hagerstown, Md., 1916*

APANTESIS VITTATA

Date.	Number taken.	Condition of ovaries.		Number and development of eggs.
		Spent.	Gravid.	
July 26	2	0	2	11D; 93D.
30	3	0	3	116D; 96D; 123D.
31	2	0	2	67D; 70D.
Aug. 3	6	0	6	129D; 146D; 122D; 131D; 96D; 224D.
4	5	0	5	78D; 89D; 108D; 95D; 113D.
6	3	0	3	77D; 53D; 47D.
8	1	0	1	103D.
9	1	0	1	139D.
15	1	0	1	166D.
22	1	0	1	113D.

APANTESIS ARGE

July 20	1	0	1	156D.
26	1	0	1	97D.
Aug. 25	1	0	1	129D.

TABLE III.—*Condition of the ovaries of Lepidoptera taken at a light trap, Hagerstown, Md., 1916*—Continued

ESTIGMENE ACRAEA

Date.	Number taken.	Condition of ovaries. Spent.	Gravid.	Number and development of eggs.
June 23	1	1	0	
29	1	0	1	187D.
July 1	1	0	1	98D.
2	1	0	1	117D.
14	1	1	0	
27	1	0	1	203D.
30	1	0	1	128D.
31	7	0	7	All fully developed but not counted.
Aug. 1	1	0	1	153D.
3	5	0	5	All fully developed but not counted.
4	1	1	0	
5	5	0	5	Do.
6	1	1	0	
8	3	1	2	147D; 138D.
9	1	1	0	
10	2	1	1	186D.
11	1	0	1	227D.
13	1	0	1	236D.
17	2	0	2	All fully developed but not counted.
18	3	1	2	Do.
19	3	1	2	Do.
20	3	1	2	Do.
21	4	0	4	Do.
22	3	0	3	Do.
23	5	0	5	Do.
24	5	0	5	Do.
25	4	0	4	Do.
28	2	0	2	Do.

DIACRISIA VIRGINICA

Date.	Number taken.	Spent.	Gravid.	Number and development of eggs.
June 28	2	0	2	238D; 609D.
July 2	1	0	1	514D.
24	1	0	1	488D.
28	1	0	1	378D.
29	1	0	1	471D.
31	1	0	1	538D.
Aug. 6	1	0	1	493D.

ISIA ISABELLA

Date.	Number taken.	Spent.	Gravid.	Number and development of eggs.
July 30	3	0	3	217D; 165D; 126D; 155D.
31	5	0	5	287D; 175D; 214D; 391D; 393D.
Aug. 1	2	0	2	257D; 218D.
2	1	0	1	212D.
3	1	0	1	223D.
4	7	0	7	Averaged 252D.
5	7	0	7	Averaged 217D.
6	1	0	1	158D.
8	2	0	2	164D; 192D.
9	3	0	3	387D; 320D; 392D.

TABLE III.—*Condition of the ovaries of Lepidoptera taken at a light trap, Hagerstown, Md., 1916*—Continued

ISIA ISABELLA—continued

Date.	Number taken.	Condition of ovaries. Spent.	Gravid.	Number and development of eggs.[a]
Aug. 10	1	0	1	129D.
19	1	0	1	229D.
21	1	0	1	274D.
22	3	0	3	287D; 278D; 239D.
23	1	0	1	285D.
24	1	0	1	377D.
25	1	0	1	248D.
Sept. 1	1	0	1	116D.

HALISIDOTA TESSELLARIS

July 1	4	1	3	137D; 218D; 186D.
2	3	0	3	298D; 150D(e); 200D(e).
3	13	0	13	293D; 68D; 98D; 128D; 9 averaged 167D.
4	9	0	9	5 averaged 257D; 4 averaged 137D.
6	4	0	4	168D; 157D; 128D; 92D.
7	6	0	6	128D; 153D; 4 averaged 206D.
8	21	0	21	6 averaged 260D; 15 averaged 180D.
10	4	0	4	178D; 58D; 100D(e); 75D(e).
14	2	0	2	280D; 124D.
18	6	0	6	278D; 238D; 217D; 3 averaged 250D(e).
19	4	0	4	283D; 3 averaged 225D(e).
20	10	1	9	213D; 119D; 183D; 198D; 5 averaged 180D(e).
23	10	2	8	128D; 58D; 67D; 5 averaged 110D(e).
24	1	0	1	139D.
26	3	0	3	296D; 200D (e); 225D (e).
28	10	0	10	134D; 129D; 96D; 125D; 164D; 5 averaged 105D.
29	2	1	1	108D.
31	1	0	1	143D.
Aug. 1	3	0	3	195D; 97D; 128D.
4	1	0	1	238D.
6	1	0	1	173D.
7	1	0	1	143D.
18	4	0	4	106D; 88D; 118D; 138D.

DATANA MINISTRA

July 23	7	0	7	Averaged 248D.
24	4	0	4	81D; 172D; 93D; 125D.
26	1	0	1	81D.
29	1	0	1	76D.
30	3	0	3	91D; 141D; 224D.
Aug. 1	1	0	1	6D.
3	1	0	1	263D.
4	1	0	1	332D.

[a] (e)=Estimated.

TABLE III.—*Condition of the ovaries of Lepidoptera taken at a light trap, Hagerstown, Md., 1916*—Continued

ARSILONCHE ALBOVENOSA

Date.	Number taken.	Condition of ovaries. Spent.	Condition of ovaries. Gravid.	Number and development of eggs.
July 4	3	0	3	157S; 173S; 186S.
10	2	0	2	377D; 272D.
13	1	0	1	182S.
Aug. 17	1	0	1	357—198D and 159S.
19	2	0	2	315—153D and 162S; 52D; 162S.
23	2	0	2	213D; 263D.

AUTOGRAPHA BILOBA

Date.	Number taken.	Spent.	Gravid.	Number and development of eggs.
July 4	1	1	0	
Aug. 15	1	1	0	

AUTOGRAPHA SIMPLEX

Date.	Number taken.	Spent.	Gravid.	Number and development of eggs.
June 23	15	5	10	Immature; not counted.
28	3	0	3	78D; 113D; 67D.
29	1	0	1	151D.
July 1	9	3	6	Averaged 123D.
2	7	2	5	Averaged 146D.
4	1	1	0	
6	6	0	6	Averaged 92D.
7	6	0	6	Averaged 79D.
8	12	4	8	Averaged 88D.
27	1	1	0	
30	1	0	1	128D.
Aug. 1	1	0	1	145D.
3	1	1	0	
4	2	2	0	
8	3	0	3	78D; 75D; 75D.
Oct. 4	2	0	2	156D; 168D.

MELIANA DIFFUSA

Date.	Number taken.	Spent.	Gravid.	Number and development of eggs.
June 23	1	1	0	
29	3	2	1	75D.
July 19	1	0	1	78D.
Aug. 9	1	0	1	54D.
10	2	0	2	59D; 64D.
16	1	0	1	79D.
20	1	1	0	
21	2	2	0	
24	2	2	0	
25	1	0	1	79D.
28	1	1	0	
31	1	1	0	
Sept. 2	2	2	0	

TABLE III.—*Condition of the ovaries of Lepidoptera taken at a light trap, Hagerstown, Md., 1916*—Continued

POLIA RENIGERA

Date.	Number taken.	Condition of ovaries.		Number and development of eggs.[a]
		Spent.	Gravid.	
June 23	11	1	10	Averaged 60D.
28	6	2	4	63D; 42D; 53D; 68D.
29	22	3	19	Averaged 52D.
July 1	8	0	8	Averaged 73D.
3	12	2	10	Averaged 39D.
6	3	1	2	23D; 75D.
8	4	0	4	51D; 28D; 43D; 73D.
10	2	0	2	32D; 43D.
24	1	0	1	38D.
Aug. 22	1	0	1	43D.
23	7	1	6	Averaged 57D.

CAENURGIA ERECHTEA

Date.	Number taken.	Spent.	Gravid.	Number and development of eggs.[a]
June 28	8	2	6	4 averaged 42D; 125S(e); 150S(e).
29	11	4	7	Averaged 51D.
July 1	39	13	26	16 averaged 53D; 10 averaged 125S(e).
2	18	7	11	6 averaged 29D; 5 averaged 125S(e).
3	63	21	42	30 averaged 62D; 12 averaged 130S.
4	20	8	12	9 averaged 44D; 3 averaged 110S.
6	38	10	28	25 averaged 47D; 3 averaged 150S(e).
7	44	12	32	30 averaged 45D; 2, 125S each (e).
8	97	49	48	40 averaged 57D; 8 averaged 150S(e).
10	7	2	5	Averaged 43D.
14	2	0	2	26D; 21D.
18	5	3	2	23D; 26D.
19	11	6	5	Averaged 28D.
20	12	4	8	Averaged 31D.
23	17	7	10	Averaged 51D.
24	6	3	3	27D; 54D; 100D.
26	6	2	4	Averaged 104D.
28	1	0	1	86D.
29	1	1	0	
30	4	4	0	
Aug. 1	1	0	1	21D.
2	1	1	0	
3	10	5	5	Averaged 59D.
4	3	2	1	19D.
7	1	0	1	34D.
8	12	5	7	Averaged 55D.
9	6	5	1	33D.
10	44	23	21	18 averaged 32D; 3 averaged 130S(e).
12	2	1	1	6D.
13	1	1	0	
14	3	3	0	
15	13	7	6	75D; 5 averaged 22D.
16	5	2	3	16D; 31D; 23D.
17	18	7	11	8 averaged 53D, 3 averaged 130S(e).
18	12	7	5	4 averaged 43D; 175S(e).
19	10	6	4	Averaged 25D.
20	9	5	4	Averaged 44D.
21	23	11	12	9 averaged 46D; 3 averaged 150S(e).
22	31	20	11	10 averaged 42D; 1, 125S(e).
23	62	36	26	19 averaged 40D; 7 averaged 125S(e).
24	38	24	14	10 averaged 43D; 4 averaged 110S(e).

[a] (e)—Estimated.

TABLE III.—*Condition of the ovaries of Lepidoptera taken at a light trap, Hagerstown, Md., 1916*—Continued

CAENURGIA ERECHTEA—continued

Date.		Number taken.	Condition of ovaries. Spent.	Gravid.	Number and development of eggs.[a]
Aug.	25	40	22	18	15 averaged 36D; 3 averaged 75S(e).
	28	3	1	2	24D; 32D.
	30	24	13	11	9 averaged 34D, 75S(e); 100S(e).
	31	24	14	10	Averaged 30D.
Sept.	1	13	3	10	Averaged 31D.
	2	5	4	1	23D.
	14	6	1	5	Averaged 51D.
	18	1	1	0	
Oct.	6	2	1	1	22D.

CAENURGIA CRASSIUSCULA

Date.		Number taken.	Spent.	Gravid.	Number and development of eggs.
June	28	4	1	3	96D; 108D; 57D.
	29	4	2	2	53D; 28D.
July	1	19	7	12	8 averaged 53D; 4 averaged 125S(e).
	2	9	3	6	3 averaged 29D; 3 averaged 125S(e).
	3	29	10	19	11 averaged 62D; 8 averaged 130S(e).
	4	10	4	6	3 averaged 44D; 3 averaged 110S.
	6	21	6	15	13 averaged 47D; 2, 150S(e).
	7	22	6	16	13 averaged 45D; 3 averaged 125S(e).
	8	51	29	22	17 averaged 51D; 5 averaged 150S(e).
	10	4	1	3	29D; 43D; 21D.
	14	2	1	1	47D.
	18	4	0	4	Averaged 25D.
	19	6	3	3	Averaged 67D.
	20	9	7	2	19D; 16D.
	23	3	1	2	81D; 56D.
	24	1	1	0	
	26	3	2	1	128S.
Aug.	1	1	1	0	
	2	2	2	0	
	3	5	3	2	100D; 25D.
	6	2	2	0	
	8	6	3	3	21D; 100S(e); 100S(e).
	9	5	4	1	75D.
	10	13	8	5	3 averaged 32D; 100S(e); 100S(e).
	12	3	3	0	
	13	2	0	2	41D; 31D.
	14	5	5	0	
	15	24	13	11	8 averaged 22D; 3 averaged 75S.
	16	8	4	4	Averaged 31D.
	17	20	9	11	7 averaged 53D; 4 averaged 130S(e).
	18	23	12	11	8 averaged 43D; 3 averaged 100S(e).
	19	19	11	8	Averaged 38D.
	20	17	9	8	Averaged 44D.
	21	25	13	12	10 averaged 40D; 125S(e); 175S(e).
	22	68	40	28	22 averaged 42D; 6 averaged 125S(e).
	23	63	37	26	20 averaged 40D; 6 averaged 125S(e).
	24	13	6	7	6 averaged 43D; 125S(e).
	25	15	9	6	5 averaged 36D; 100S(e).
	28	2	1	1	64D.
	30	12	6	6	5 averaged 34D; 75S.
	31	8	4	4	Averaged 30D.
Sept.	2	3	1	2	42D; 28D.
	14	1	0	1	75D.

[a] (e)=Estimated.

TABLE III.—*Condition of the ovaries of Lepidoptera taken at a light trap, Hagerstown, Md., 1916*—Continued

CIRPHIS UNIPUNCTA

Date.	Number taken.	Condition of ovaries. Spent.	Condition of ovaries. Gravid.	Number and development of eggs.[a]
June 28	4	0	4	578D; 539D; 550D; 575D.
29	3	0	3	638D; 619D; 543D.
July 1	19	6	13	118D; 483D; 648D; 10 averaged 475D(e).
2	7	1	6	397D; 378D; 586D; 3 averaged 400D(e).
3	42	10	32	4 averaged 362D; 28 averaged 375D(e).
4	13	3	10	523D; 618D; 703D; 600D(e); 550D(e); 525D(e); 575D(e); 500D(e); 525D(e); 325D(e).
6	13	1	12	638D; 679D; 587D; 9 averaged 535D(e).
7	90	11	79	128D; 383D; 744D; 773D; 75 averaged 525D(e).
8	57	21	36	625D; 587D; 634D; 718D; 32 averaged 365D(e).
10	22	5	17	625D; 473D; 587D; 713D; 13 averaged 505D(e).
18	10	2	8	713D; 628D; 478D; 5 averaged 475D(e).
19	14	3	11	554D; 623D; 478D; 8 averaged 512D(e).
20	12	0	12	657D; 713D; 538D; 9 averaged 530D(e).
23	19	8	11	107D; 78D; 576D; 8 averaged 565D(e).
24	2	2	0	
27	6	2	4	663D; 587D; 713D; 629D.
28	8	1	7	684D; 593D; 567D; 4 averaged 550D(e).
29	3	0	3	567D; 627D; 493D.
30	5	0	5	692D; 563D; 478D; 450D(e); 525D(e).
31	4	1	3	613D; 576D; 550D(e).
Aug. 1	4	0	4	273D; 438D; 557D; 425D.
2	2	0	2	397D; 453D.
3	15	2	13	625D; 583D; 518D; 10 averaged 477D(e).
4	11	0	11	576D; 487D; 682D; 8 averaged 528D(e).
6	5	0	5	718D; 700D; 475D; 525D; 375D.
7	1	0	1	378D.
8	6	0	6	486D; 726D; 4 averaged 550D(e).
9	7	0	7	623D; 6 averaged 495D(e).
10	10	3	7	387D; 658D; 5 averaged 480D(e).
11	5	1	4	487D; 633D; 550D(e); 425D(e).
16	1	1	0	
19	1	0	1	563D.
22	2	0	2	432D; 328D.
Sept. 27	1	1	0	

NOCTUA C-NIGRUM

Date.	Number taken.	Spent.	Gravid.	Number and development of eggs.
Aug. 8	5	3	2	Fully developed; not counted.
9	13	10	3	Do.
10	7	5	2	Do.
11	2	2	0	
14	1	0	1	Do.
15	1	0	1	Do.
18	7	2	5	Do.
21	2	1	1	Do.
22	11	7	4	Do.
23	15	8	7	Do.
24	13	6	7	Do.
25	7	3	4	Do.
30	7	3	4	218D; 198D; 238D; 1 not counted.
31	5	2	3	Fully developed; not counted.
Sept. 1	1	1	0	

[a] (e)=Estimated.

TABLE III.—*Condition of the ovaries of Lepidoptera taken at a light trap, Hagerstown, Md., 1916*—Continued

NOCTUA C-NIGRUM—continued

Date.	Number taken.	Condition of ovaries.		Number and development of eggs.[a]
		Spent.	Gravid.	
Sept. 2	2	0	2	Fully developed; not counted.
14	2	2	0	
18	3	1	2	Do.
Oct. 6	3	2	1	Do.

FELTIA SPP.

Date.	Number taken.	Spent.	Gravid.	Number and development of eggs.[a]
Aug. 8	4	0	4	200D(e); 200D(e); 300S(e); 300S(e).
9	3	1	2	69D; 198D.
10	3	1	2	154D; 300S(e).
12	1	0	1	43D.
14	1	0	1	233D.
15	3	1	2	73D; 250S(e).
17	1	0	1	432S.
18	1	0	1	254D.
19	1	0	1	238D.
20	3	0	3	101D; 154D; 298D.
21	14	1	13	78D; 158D; 149D; 98D; 228S; 4 averaged 288S; 4 averaged 213D.
22	43	10	33	7 averaged 163D; 22 averaged 112D; 4 averaged 250S(e).
23	38	5	33	7 averaged 117D; 20 averaged 130D(e); 6 averaged 258S(e).
24	38	10	28	112D; 183D; 74D; 81D; 218D; 228S; 258S; 16 averaged 113D(e); 5 averaged 230S(e).
25	65	20	45	10 averaged 120D; 5 averaged 277S; 21 averaged 125D(e); 9 averaged 293S(e).
28	8	2	6	258D; 128D; 178D; 151D; 200S(e); 200S(e).
30	75	16	59	128D; 78D; 64D; 159D; 235S; 346S; 271S; 43 averaged 115D(e); 9 averaged 225S(e).
31	57	32	25	128D; 174D; 64D; 152D; 137D; 154D; 176D; 8 averaged 120D(e); 10 averaged 219S(e).
Sept. 1	33	6	27	221D; 153D; 186D; 131D; 74D; 68D; 18 averaged 154D(e); 200S(e); 200S(e); 468S.
2	26	6	20	78D; 153D; 47D; 53D; 5 averaged 100D; 11 averaged 136D(e).
4	11	10	1	141D.
5	10	9	1	128D.
7	11	7	4	18D; 209D; 74D; 199D.
14	127	91	36	236D; 158D; 95D; 101D; 128D; 86D; 30 averaged 94D(e).
18	54	47	7	196D; 156D; 116D; 76D; 226S; 125D(e); 50D(e).
27	37	29	8	86D; 180D; 76D; 226D; 4 averaged 88S(e).
28	85	48	37	96D; 126D; 233D; 76D; 56D; 74D; 101D; 30 averaged 120D(e).
Oct. 6	45	42	3	219D; 48D; 54D.

[a] (e)=Estimated.

The one species (*Datana ministra*) of the Notodontidae is represented by 19 females, all of which were gravid.

Among the Noctuidae all the females of one species (*Arsilonche albovenosa*) were found to be gravid. One species, *Autographa biloba*, is

represented by only 2 females, both spent. Of the remaining species of this family the percentage of gravid females varies from 37 per cent in *Meliana diffusa* to 87 per cent in *Polia renigera*. Of the 424 females of *Cirphis unipuncta* dissected, 80 per cent were gravid, the eggs ranging in number from 107 to 773, all fully developed.

Some explanation is required as to the method of arriving at the number of eggs accredited to a female moth where a footnote to a table reads "Estimated." The ovarian structure was dissected and spread for counting the eggs, adopting a unit of 25 eggs. The remaining ovarian material was divided into masses of the bulk of that containing 25 eggs. This method was frequently verified by actual counts and it is believed that the figures are dependable. Where no such reference appears, the actual count was made. In every case the stage of development was determined under the hand lens or binocular and indicated in Table III by "D" for "developed" and by "S" for "immature."

Any data as to the relative proportions of male and female Lepidoptera taken at a light trap have an added value when considered in connection with information bearing on these relations of the sexes in nature. For this reason the writer has endeavored to get together all facts to be had from available sources, and brief notes on the subject are cited here under the name of the species concerned.

EUPROCTIS CHRYSORRHOEA LINNAEUS (*3, p. 47–48*)[1]

Concerning the brown-tail moth Fernald and Kirkland write as follows:

> In July, 1897, a quantity of cocoons and pupæ was gathered and placed in a large glass-covered box, the moths being removed as they emerged. The following . . . shows the relative proportion of the sexes: Males, 399; females, 451.

ELASMOPALPUS LIGNOSELLUS ZELLER (*10, p. 20*)

Records obtained at Columbia, S. C., in 1915. From 56 pupæ there emerged 23 males and 33 females.

PHTHORIMAEA OPERCULELLA ZELLER (*4, p. 24*)

Graf records the following data with regard to the proportion of sexes of the potato-tuber moth:

> The proportion of the sexes during the year remains very nearly constant and almost equal. Pupæ selected at random at various times of the year gave the results shown in Table 3. (327 males, 284 females.)

CRAMBUS HORTUELLUS HUEBNER (*15, p. 8*)

With regard to the cranberry girdler, Scammell records the following data:

> In the early summer the males and females appear to be about equal in number; for example, on June 11, 24 moths were collected, of which 12 were males and 12 females. In late summer, however, the males are far in excess of the females, as shown by the following collections: Thirteen moths taken July 27 consisted of 11 males and 2 females, while of 23 moths collected August 10 only 5 were females.

[1] Reference is made by number (italic) to "Literature cited," p. 148–149.

PLUTELLA MACULIPENNIS CURTIS (*11, p. 5*)

Information as to the proportional relations of the two sexes in this species is not particularly definite in the paper by Mr. Marsh, his statement being: "Fifty-two adults, about equally divided as to sex, developed on November 2 and 3." In the summing up of such data as the writer has been able to assemble, this species appears in Table XX as 26 males and 26 females.

CARPOCAPSA POMONELLA LINNAEUS (*16*)

A general deduction from all data given of rearings puts the proportional relations of the sexes as nearly equal, with a very slight preponderance of females. The same species (*5, p. 52*) is reported by Mr. A. G. Hammar as including 456 males and 563 females in a total of 1,019 individuals. Further information as to the codling moth is to be found in the paper by Messrs. Jones and Davidson (*9, p. 120–121*), where, in Table VI, the moths issuing from 151 pupæ are shown to comprise 67 males and 84 females. In Table XXIX (*9, p. 146*), of 65 adults 32 are reported as males, 33 as females, while in Table XL (*9, p. 153*) the males make up only 21 of a total of 54. Summing up the data for *C. pomonella* it is found that of 1,289 individuals the males include 576; the females 713; a percentage of 44.7 and 55.3, respectively.

SANNINOIDEA OPALESCENS HENRY EDWARDS (*12, p. 79*)

Mr. Dudley Moulton in his records for 1908 and 1909 on this species accounts for 232 adults and lists them as 118 males, 114 females.

SYNANTHEDON PICTIPES GROTE AND ROBINSON (*8, p. 411*)

Mr. J. L. King, in his paper on the lesser peach-tree borer, places 12 adults as to sex; 4 are determined as males and 8 as females. On the same page of the bulletin five adults are divided as to sex into 2 males and 3 females.

ARCHIPS ARGYROSPILA WALKER (*6, p. 257*)

Messrs. Herrick and Leiby had under observation 227 pupæ from larvæ kept in jars "in an open air insectary under normal conditions of temperature." Sex determinations of 155 individuals proved 85 to be males and 70 to be females.

The same species was under observation by Mr. W. M.. Davidson (*2*) in 1911, who states that of 76 adults 29 were males and 47 were females.

ARCHIPS ROSACEANA HARRIS (*14, p. 396*)

In an article by E. D. Sanderson and Mrs. A. D. Jackson, published in the Journal of Economic Entomology, December, 1909, the authors state that from 62 pupæ there issued 35 males and 27 females.

HALISIDOTA CARYAE HARRIS (*7, p. 8*)

Mr. Dwight Isely had this species under observation at North East, Pa., during the summers of 1915 and 1916. He records that of 25 adults reared 17 were males and 8 were females.

CHLORIDEA OBSOLETA FABRICIUS (*13, p. 92*)

Of this species it is stated that—

. . . data concerning over 300 moths were collected which bear evidence on the proportions of the sexes. These include records of moths collected in the field and of those bred out in the laboratory. In practically all cases there is a slight preponderance of females in the ratio of 168 females to 120 males.

HEMILEUCA OLIVIAE COCKERELL (*1, p. 84, 88*)

In his paper on this species Mr. C. N. Ainslie says:

During the first week of emergence the males outnumbered the females at least three to one, and on page 88 a table shows that from 5,000 pupæ gathered in widely separated parts of the infested area there emerged 2,822 males as against 2,178 females.

Further information concerning this species is had from manuscript records on the relative proportions of the sexes, compiled from pupal parasite cages at Koehler, N. Mex., by Messrs. V. L. Wildermuth, D. J. Caffrey, and H. E. Smith, during September, October, and November, 1913. These records concern a total of 19,321 moths, of which 10,844 were males and 8,477 were females.

PORTHETRIA DISPAR LINNAEUS

Under date of December 15, 1917, Mr. F. H. Mosher, Entomological Assistant, states that of the large number of gipsy moths reared in investigations extending over a period of six years the ratio of males to females averaged as 5 to 4, a percentage of 55.6 and 44.4, respectively.

A summing up of the foregoing notes on the proportional relations of the sexes in the Lepidoptera is presented in Table IV, by which it is seen that of 28,094 individuals, the males make up 55 per cent and the females 45 per cent Although 14 species are concerned, the bulk of moths are of one species, *Hemileuca oliviae*. It is to be regretted that the matter of the proportion of sexes among Lepidoptera has received so little attention.

If it be assumed that the sexes exist in nature in approximately equal numbers, the investigations on which this paper is based show the females taken at the light trap to constitute 57 per cent of the assumed total of females, while the gravid females so taken make up 33 per cent. It is believed that further investigations to be conducted will adduce additional evidence to disprove the theory that practically only male

Lepidoptera are attracted to light traps and that of the females so captured all have previously oviposited.

TABLE IV.—*Summary of foregoing records, compiled mainly from the literature, as to the relative proportions of male and female Lepidoptera*

Species.	Number of males.	Number of females.	Total.	Percentage of males.	Percentage of females.
Euproctis chrysorrhoea Linnaeus	399	451	850	47	53
Elasmopalpus lignosellus Zeller	23	33	56	41	59
Phthorimaea operculella Zeller	327	284	611	53.5	46.5
Crambus hortuellus Hübner	41	19	60	68.3	31.7
Plutella maculipennis Curtis	26	26	52	50	50
Carpocapsa pomonella Linnaeus	576	713	1,289	44.7	55.3
Hemileuca oliviae Cockerell	13,666	10,655	24,321	56	44
Sanninoidea opalescens Henry Edwards	118	114	232	50.9	49.1
Synanthedon pictipes Grote and Robinson	6	11	17	35.3	64.7
Archips argyrospila Walker	114	117	231	49.4	50.6
Archips rosaceana Harris	35	27	62	56.5	43.5
Halisidota caryae Harris	17	8	25	68	32
Chloridea obsoleta Fabricius	120	168	288	42	58
Porthetria dispar Linnaeus	......	......	......	55.6	44.4

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A COMPARATIVE STUDY OF SALT REQUIREMENTS FOR YOUNG AND FOR MATURE BUCKWHEAT PLANTS IN SOLUTION CULTURES

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INTRODUCTION

Since the general recognition of the fact that the mineral elements essential to plant growth are derived from the soil solution and not, as suggested by Liebig, directly from the soil particles, culture solutions have assumed a very important rôle in the investigation of problems relating to plant nutrition. The first standard nutrient solution for plants was proposed by Sachs (5)[1] in 1860. Since then a number of formulas for the preparation of standard nutrient solutions have been suggested by different investigators. Many of the standard solutions now in common use have been recommended as producing good growth of various kinds of plants, without reference to any particular stage of development in the life cycle of the plant, for which the solutions are best adapted. It is entirely possible, however, that in a culture solution the relative proportions of the mineral constituents required to produce good growth of a given species during one physiological period might be entirely different from the proportions of the same constituents required to maintain equally good growth during another developmental period. A nutritive medium which is capable of producing good seedlings rooted in it might not be at all suitable for sustaining the development of the same plants with the approach of maturity nor for producing seed. On the other hand, a nutritive solution or other medium which is well adapted to the growth of mature plants might actually be injurious to the seedlings of the same species.

The nutrient medium recommended by Tottingham (*8*) and the 3-salt solution later proposed by Shive (*6*) are known to produce excellent growth of wheat seedlings during the first three or four weeks of development after germination. Whether these solutions are capable of sustaining the growth of wheat plants equally well throughout their entire life period, has not been determined.

[1] Reference is made by number (italic) to "Literature cited," p. 175.

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As the result of extensive comparative studies with both wheat (*Triticum* spp.) (6) and buckwheat (*Fagopyrum esculentum*) (7), it has been found that, under certain experimental conditions and for a period of growth of about four weeks directly following germination, 3-salt solutions in suitable concentrations and with the proper proportions of the three salts, monopotassium phosphate, calcium nitrate, and magnesium sulphate (with a trace of iron added), are equal in plant-producing power to any of the more complex solutions now in common use. It seemed highly desirable to determine whether the salt proportions demanded for approximately optimum development during the later periods of growth, for the maturing of the plants, and for seed production are the same or different from those which produce the best growth during the early stages of development. An attempt has been made in this direction, and the present paper describes an experimental study of the salt requirements of buckwheat plants in water cultures, for the later periods of development from the flowering stage to the maturing of seeds. These results are compared with those of a similar study (7), previously carried out, of the development of young buckwheat plants during the growth period from germination to the flowering stage. A similar comparative study of salt requirements for buckwheat has been carried out with sand cultures. The results with water cultures will alone be presented in the following pages; those obtained with sand cultures will be reserved for later publication.

Buckwheat was chosen for these tests for the reason that in its life cycle it presents two distinct physiological growth periods which extend over nearly equal periods of time. The first of these occupies the period between the germination of the seeds and flowering, the second interval extending over the period from the flowering stage to the maturity of the seeds. It is a quick-growing plant and matures in a comparatively brief period of time, requiring, under favorable conditions, about 60 days to complete its active growing period.

EXPERIMENTAL PROCEDURE AND METHODS

The tests to be described in the following pages were carried out with the optimal series of 3-salt solutions [1] previously employed by Shive in his work with wheat and with buckwheat. This optimal series comprised 36 different solutions, all having approximately the same total osmotic concentration value of 1.75 atmospheres. The three salts were so distributed as to include all possible sets of proportions of the three salts when the partial concentrations of the three components were made to vary by equal increments of one-tenth of the total osmotic concentration. To each solution was always added the usual trace of iron, in the form of ferric phosphate.

[1] A table of these solution formulas has been given in previous publications: Shive, J. W. (6), and McCall, A. G. (5). A discussion of the methods of calculation by which the partial osmotic concentration values and also the volume-molecular partial concentrations of each salt in these solutions may be calculated, is given by Tottingham (8, *p. 177–182, 192*).

In the work previously carried out with buckwheat (7), tests of the 36 different sets of proportions of the three salts of this series showed that the best growth of tops and of roots during the early physiological growth period extending from germination to the flowering stage was produced by a solution containing the three salts in the following volume-molecular proportions: Potassium phosphate (KH_2PO_4), 0.0144 m; calcium nitrate [$Ca(NO_3)_2$], 0.0052 m; and magnesium sulphate ($MgSO_4$), 0.0200 m. This solution was further characterized by having four-tenths of its total osmotic concentration derived from potassium phosphate, four-tenths from calcium nitrate, and two-tenths from magnesium sulphate.

The methods [1] employed with the water cultures here considered were similar to those previously adopted in the work with wheat and with buckwheat. The Japanese variety of buckwheat was used. The seedlings were carefully selected for uniformity of size and vigor, and when about 5 cm in height were transferred to the culture vessels, which consisted of pint Mason jars. Each culture vessel had a capacity of 515 cc. Three buckwheat plants were included in each culture.

In order to determine the proportions of the three salts required to produce approximately optimum growth of buckwheat plants during the later period of development, from the flowering stage to the maturing of the seed, it was of course essential that the plants of all the cultures at the beginning of this later period of growth should be as nearly uniform as possible. With this in view, many more cultures than the 36 comprising the series for study were prepared at the same time with selected seedlings all nearly uniform in size and vigor. The selected seedlings were transferred to the culture vessels, each of which had previously been provided with 515 cc of the same solution. This solution, with a total osmotic concentration value of 1.75 atmospheres, had the salt proportions above given. These produced the highest dry weight yield of buckwheat tops and of roots during the early growth period, between germination and the beginning of the flowering stage. All the seedlings were grown in this solution, with renewal of solutions every four or five days during the first 24-day growth period after the seedlings had been transferred to the solutions. At the end of this time period the plants of each culture had begun to bloom and all the plants appeared healthy and vigorous, the cultures throughout showing excellent uniformity.

Thirty-six cultures were now selected from the larger number at hand; these were transferred to the 36 different solutions comprised in the optimal 3-salt series. All the solutions of the series had a total osmotic concentration value of approximately 1.75 atmospheres, but they differed from each other in the proportions of the component salts. For purposes of comparison, one culture was also transferred to Knop's

[1] For a description of the methods employed in these studies, see Shive, J. W. (6).

solution and another to Tottingham's best solution for wheat tops, each with a total concentration value equal to that of the 3-salt solutions. Thus, the actual tests of the effects of the 36 different salt combinations upon the growth of the plants in this series, were not begun until the plants had passed through the first physiological growth period extending from the germination of the seed to the flowering stage. The cultures were now continued, with renewal of solutions as before, until the seeds were mature. This required 28 days. The entire active growth period of the plants of this series extended, therefore, over an interval of 52 days after the seedlings had been transferred to the culture vessels. The series was then repeated. The second series was carried out in the same manner as the first, but under somewhat different seasonal conditions.

In order that all the plants might be exposed to somewhat similar changes of temperature, light, and moisture, the cultures were arranged in rows on a central table in the greenhouse, in such a manner as to avoid, as far as possible, unequal shading of one culture by another. Throughout the growth period the cultures were shifted in position at regular intervals, in accordance with a definite plan.

As previously stated, the culture solutions were renewed at intervals of four or five days. At the time of each renewal of solutions, the absorption (and approximately the transpirational water loss) was determined by measuring the volume of the used solution before discarding it, and subtracting this volume from the original volume (515 cc). The total transpirational water loss for each culture was obtained by summing the losses for the partial periods between each two successive changes of solution.

At the end of the growth period, after practically all the seeds were ripe, the plants were harvested. The tops were separated from the roots just above the topmost lateral root. The seeds were carefully removed from the tops, and the three portions were separately dried to constant weight at a temperature of about 103° C. The dry weights were then obtained.

Records were kept of the temperature in the greenhouse where the culture series were conducted. Daily maximum and minimum temperature readings were obtained from thermometers protected from direct sunlight. The moisture conditions of the atmosphere throughout the growth periods were indicated by means of the evaporation rates from spherical porous-cup atmometers. Several of these instruments were placed among the cultures on the greenhouse table, and daily readings were taken. These were corrected to the Livingston (*2*) standard spherical atmometer.

EXPERIMENTAL RESULTS

The first of the two culture series carried out during the later developmental growth period (between the flowering stage and the ripening of

the seeds) was conducted from October 18 to December 9, 1916. During this period the maximum temperature recorded was 30° C., on November 3, and the minimum was 11° on December 4. The rate of evaporation from the atmometer gave a daily mean of 16.8 cc, a maximum daily rate of 25.6 cc, on November 21, a minimum daily rate of 7.4 cc, on October 20, and a total water loss from the instrument of 874 cc. The second series of cultures, which was just like the first, extended over the period from December 9, 1916, to January 30, 1917. During this period a maximum temperature of 30° occurred on December 28, and a minimum of 7° on January 1. The rate of water loss from the porous-cup atmometer, indicating the evaporating power of the air gave a daily mean of 17.7 cc, a maximum daily rate of 25.2 cc, on January 13, and a minimum daily rate of 13.5 cc, on January 29. The total loss from the instrument for the entire time was 919 cc.

In the following sections, the results obtained with these buckwheat cultures grown from the flowering stage to maturity in an optimal series of 3-salt solutions with their different sets of salt proportions will be compared with those obtained from a similar study (7) previously carried out with buckwheat grown in the solution cultures of the same series, but conducted only to the flowering stage, a period of about four weeks directly after germination. The comparisons will be made with reference to the dry weights of tops and of roots and also with respect to the relative amounts of water lost by transpiration during the growth periods.

I.—DRY WEIGHTS

A.—Presentation of Data

The tops, roots, and seeds of the cultures grown to maturity were weighed separately. Three sets of dry-weight measurements are therefore available. Since the results obtained with the two corresponding series were in very close agreement, only average dry-weight yields will here be considered. These are presented in Table I. In every case these measurements represent the values obtained by averaging the corresponding data of the two series conducted during different time periods. In the first column are given the culture numbers. These refer to the positions which the cultures occupy on the triangular diagram graphically representing the variations in the salt proportions and partial osmotic concentrations of the series of solution cultures here employed. Since the scheme of diagramatic representation for this series of solution cultures has been explained in a previous publication (*6, p. 341*) and has since been employed by McCall (*3, 4*), the description of the diagram may here be omitted. Table I gives the average absolute dry weights, in grams, of tops, roots, and seeds, and also the dry-weight values in terms of the corresponding value of culture R1C1 considered as unity. These relative yields were obtained by dividing the average absolute dry-weight value of each culture by the corresponding value of culture R1C1. The maximum relative yields are here indicated by bold-face type. The

last two items in each column (numbered K and T, respectively) refer to the data obtained with the cultures grown in Knop's solution and in Tottingham's best solution for wheat tops, each with the same total concentration as the solutions of the 3-salt series. The cultures were included in each series for comparison.

TABLE I.—*Average dry weights of tops, roots, and seeds of buckwheat grown from the flowering stage to maturity in 3-salt solutions, all having a total osmotic concentration value of 1.75 atmospheres, but differing from each other in the proportions of the 3 salts; also the ratio of tops to seeds*

Culture No.	Average dry-weight yields.						Ratio of tops to seeds.
	Tops (3 plants).		Roots (3 plants).		Seeds (3 plants).		
	Absolute.	Relative to R1C1 as unity.	Absolute.	Relative to R1C1 as unity.	Absolute.	Relative to R1C1 as unity.	
	Gm.		*Gm.*		*Gm.*		
R1C1	1. 808	1. 00	0. 132	1. 00	0. 789	1. 00	2. 30
R1C2	2. 112	1. 17	. 176	1. 33	1. 128	1. 37	1. 65
R1C3	1. 832	1. 01	. 164	1. 24	1. 203	1. 52	1. 52
R1C4	2. 255	1. 25	. 228	1. 73	1. 838	2. 35	1. 21
R1C5	2. 311	1. 28	. 251	1. 90	1. 687	2. 14	1. 36
R1C6	2. 041	1. 13	. 187	1. 24	1. 229	1. 56	1. 66
R1C7	2. 604	1. 44	. 270	2. 05	1. 551	1. 97	1. 68
R1C8	2. 408	1. 33	. 269	2. 04	1. 493	1. 89	1. 62
R2C1	2. 013	1. 11	. 184	1. 39	. 735	. 93	2. 77
R2C2	2. 365	1. 31	. 222	1. 68	1. 009	1. 28	2. 37
R2C3	2. 279	1. 26	. 223	1. 69	1. 405	1. 78	1. 62
R2C4	2. 060	1. 14	. 203	1. 54	1. 756	2. 21	1. 17
R2C5	2. 105	1. 17	. 204	1. 82	1. 811	2. 30	1. 16
R2C6	2. 140	1. 18	. 243	1. 84	1. 488	1. 88	1. 44
R2C7	2. 210	1. 22	. 203	1. 54	1. 729	2. 20	1. 28
R3C1	2. 025	1. 12	. 201	1. 52	1. 024	1. 28	1. 99
R3C2	2. 359	1. 31	. 251	1. 90	1. 098	1. 34	2. 12
R3C3	2. 288	1. 27	. 287	2. 17	2. 004	2. 62	1. 11
R3C4	2. 372	1. 31	. 351	2. 66	1. 819	2. 30	1. 30
R3C5	3. 258	1. 80	. 363	2. 75	1. 252	1. 59	2. 61
R3C6	2. 661	1. 47	. 267	2. 02	1. 548	1. 96	1. 72
R4C1	1. 923	1. 06	. 146	1. 11	. 279	. 35	6. 89
R4C2	2. 260	1. 25	. 232	1. 76	1. 428	1. 81	1. 58
R4C3	2. 744	1. 52	. 280	2. 12	1. 035	1. 31	2. 64
R4C4	2. 373	1. 31	. 247	1. 87	1. 709	2. 26	1. 39
R4C5	2. 002	1. 10	. 232	1. 76	1. 623	2. 06	1. 24
R5C1	1. 952	1. 08	. 187	1. 42	1. 133	1. 44	1. 47
R5C2	2. 352	1. 30	. 212	1. 61	1. 582	2. 01	1. 49
R5C3	2. 200	1. 22	. 241	1. 83	1. 265	1. 72	1. 73
R5C4	2. 103	1. 16	. 207	2. 05	1. 711	2. 18	1. 23
R6C1	2. 066	1. 14	. 177	1. 34	1. 494	1. 90	1. 39
R6C2	2. 230	1. 23	. 255	1. 93	1. 443	1. 83	1. 55
R6C3	2. 288	1. 27	. 260	1. 97	1. 542	1. 96	1. 49
R7C1	2. 175	1. 21	. 191	1. 45	. 953	1. 21	2. 29
R7C2	2. 304	1. 27	. 198	1. 50	. 911	1. 15	2. 55
R8C1	2. 372	1. 31	. 225	1. 70	. 452	. 57	5. 24
K [a]	2. 343	1. 29	. 262	1. 99	1. 334	1. 78	1. 76
T [a]	2. 415	1. 33	. 369	2. 80	1. 402	1. 70	1. 73

[a] K and T represent Knop's solution and Tottingham's best solution for wheat, respectively. The data obtained from these cultures are introduced for comparison.

The relative yield of values of tops, roots, and seeds were plotted on triangular diagrams like those previously employed (7), and to which reference is made above. These diagrams represent graphically the distribution of the dry-weight yields taken directly from the proper column of averages in Table I. To facilitate the study of this distribution and to aid in making comparisons, the total range of yield values in the average series is divided into an upper one-fourth, comprising the nine cultures which produced the highest yields, a lower one-fourth, including the nine cultures giving the lowest yields, and a medium one-half, which includes the remaining cultures. These three partial ranges were outlined on the triangular diagrams to correspond to the regions of high, low, and medium yields. The areas of high yields (range of the best nine cultures) are indicated on the diagrams by small crosses, and the areas of low yields (range of the poorest nine cultures) are denoted by small circles. The position on the diagram of the culture giving the highest yield is shown by a larger cross, and that of the culture giving the lowest yield is indicated by a larger circle.

B.—Comparison of Results Obtained from Cultures Grown to Maturity With Those Obtained from Cultures Grown to the Flowering Stage

(1) DRY WEIGHTS OF TOPS

For the sake of convenience in the discussion, the culture series grown to the flowering stage (early developmental period) in the optimal series of 3-salt solutions comprising the 36 different sets of salt proportions, will be referred to as series A, while those conducted in the same series of solutions from the flowering stage to the maturity of the seed (late developmental period) will be designated as series B. The relative dry-weight data for these two average series are brought together in Table II. The second and third columns of this table present the average relative dry-weight values of tops and of roots for the various cultures of series A. In the fourth and fifth columns are given the corresponding data for series B. The actual dry weight, in grams, of culture R_1C_1 is given in parentheses directly below the relative value, so that the actual dry-weight value of any culture may be found by multiplying its relative value by the actual value of culture R_1C_1 in the same column.

TABLE II.—*Comparison of the average relative yields of tops and roots of buckwheat grown to the flowering stage with corresponding data for buckwheat grown from the flowering stage to maturity, in 3-salt solutions*

Culture No.	Series A. Average relative dry-weight yields at flowering stage.		Series B. Average relative dry-weight yields at maturity.	
	Tops.	Roots.	Tops.	Roots.
R1C1	1.00 (.528)	1.00 (.031)	1.00 (1.808)	1.00 (.132)
R1C2	.83	.98	1.17	1.33
R1C3	.79	1.07	1.01	1.24
R1C4	.60	.71	1.25	1.73
R1C5	.89	1.07	1.28	1.90
R1C6	.99	1.09	1.13	1.42
R1C7	1.00	1.18	1.44	2.05
R1C8	.84	.87	1.33	2.04
R2C1	1.03	1.15	1.11	1.39
R2C2	1.12	1.46	1.31	1.69
R2C3	.95	1.22	1.26	1.69
R2C4	1.18	1.39	1.14	1.54
R2C5	.92	1.09	1.17	1.82
R2C6	1.01	1.29	1.18	1.84
R2C7	.89	1.06	1.22	1.54
R3C1	1.07	1.07	1.12	1.52
R3C2	1.02	1.13	1.31	1.90
R3C3	.99	1.17	1.27	2.17
R3C4	.83	1.07	1.31	2.66
R3C5	1.05	1.22	**1.80**	**2.75**
R3C6	.72	.74	1.47	2.02
R4C1	1.02	1.43	1.06	1.11
R4C2	**1.34**	**1.50**	1.25	1.76
R4C3	.95	1.17	1.52	2.12
R4C4	.95	1.21	1.31	1.87
R4C5	.69	.76	1.10	1.76
R5C1	1.05	1.30	1.08	1.42
R5C2	.89	1.20	1.30	1.61
R5C3	.76	.80	1.22	1.83
R5C4	.83	.78	1.16	2.05
R6C1	.99	1.31	1.14	1.34
R6C2	.85	.92	1.23	1.93
R6C3	.66	.85	1.27	1.97
R7C1	.73	.98	1.21	1.45
R7C2	.61	.85	1.27	1.50
R8C1	.81	.91	1.31	1.70
K	.83	.95	1.29	1.99
T	1.01	1.18	1.33	2.80

The responses of the buckwheat plants to the different salt proportions of the various solutions in which they grew during the two distinct physiological growth periods here considered can best be compared by referring to the triangular diagrams of figure 1. The comparison will be made with reference to the ranges of the high and low average dry-weight values indicated by the extent of the corresponding areas of high and low yields outlined on the diagrams for the series of the two developmental periods. The average relative dry-weight data as given in Table II are here graphically represented, but the yield values are

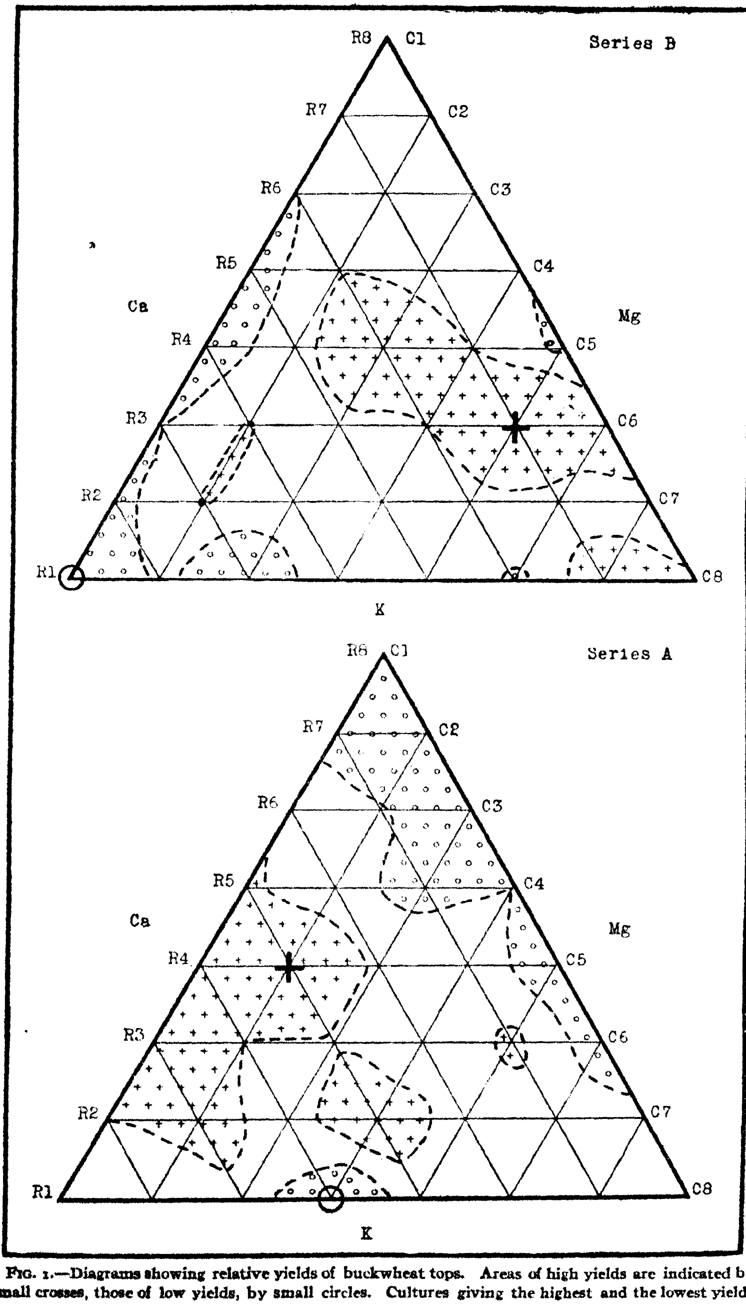

FIG. 1.—Diagrams showing relative yields of buckwheat tops. Areas of high yields are indicated by small crosses, those of low yields, by small circles. Cultures giving the highest and the lowest yields are marked by larger crosses and larger circles, respectively.

omitted to avoid confusion. The position of any culture represented on the diagrams may readily be found by means of its culture number, which always indicates the row and the number of the culture in the row. On the left margin of each diagram the rows are numbered consecutively from base to apex. The cultures of each row, represented by the points of intersection of the lines, may be considered numbered consecutively from left to right. The number of the last culture in each row is given on the right margin of the diagram. The lower triangular diagram of figure 1 represents the average yields of buckwheat tops from series A, carried out during the physiological growth period extending from germination to the flowering stage. The upper diagram represents the corresponding yields from series B, conducted from the flowering stage to the maturity of the seed.

(a) COMPARISON OF THE EFFECTS OF THE VARIOUS OSMOTIC SALT PROPORTIONS FOR THE TWO DIFFERENT PHYSIOLOGICAL GROWTH PERIODS. CONSIDERATION OF THE RELATIVE DRY WEIGHTS OF TOPS.—It will be observed that in the diagram of series A (fig. 1) the main area of low yields, including eight of the nine cultures lying within the range of low dry weights of tops, extends along the right margin to the apex of the triangular diagram, while in series B the main area of low yields borders on the left margin, extending to the base of the triangle at the lower left, and includes six cultures. It is to be noted that only a single culture (R4C5) included in the low area of series A is also included in the low area of series B. There is, moreover, with the exception of this one culture, scarcely any overlapping of the areas of low yields on the diagrams of the two series. The lowest average yields of tops do not occur with corresponding cultures of the two series. The lowest average yield produced by series A and by series B occurred with cultures R1C4 and R1C1, respectively. The former is characterized by the lowest osmotic proportions of monopotassium phosphate and medium proportions of calcium nitrate and magnesium sulphate, while the latter is characterized by the lowest proportions of monopotassium phosphate and calcium nitrate, and by the highest proportion of magnesium sulphate.

The main area of high yields on the diagram of series A occupies a central region lying to the left of the vertical axis of the diagram and includes four cultures on the left margin. Two secondary high areas are also indicated for cultures R2C4 and R3C5. On the diagram of series B the main area of high dry weights occupies a central region lying mainly to the right of the vertical axis of the diagram and extends to the right margin at culture R3C6. An outlying high area is indicated at the lower right, and a smaller one about cultures R2C2 and R3C2; but these two cultures, together with culture R8C1 at the apex of the diagram, mark the lower limit of the range of high yields. The main areas of high yields of the two series are thus seen to lie on opposite sides of the diagrams, as do also the main areas of low dry weights.

A comparison of the solutions which produced the highest average yields of tops in the two series representing the two different physiological growth periods here considered, indicates the best proportions of the salts to be markedly different for the two growth periods in question. The highest yield of tops for series A occurred with culture R4C2, which is characterized, as previously stated, by having four-tenths of its osmotic value due to monopotassium phosphate, and two-tenths and four-tenths due, respectively, to calcium nitrate and to magnesium sulphate. The yield from this culture was 34 per cent higher than the corresponding yield from culture R1C1. The best physiological balance for series B is shown for culture R3C5. This culture derived three-tenths of its total osmotic value from monopotassium phosphate, five-tenths from calcium nitrate, and two-tenths from magnesium sulphate. It produced a yield of tops which was 80 per cent higher than the corresponding yield from culture R1C1. It thus appears that the greatest production of dry weight of tops in this series of 3-salt solutions, with total osmotic concentrations of 1.75 atmospheres, and for buckwheat plants during the period of growth between the flowering stage and the ripening of the seeds, may be expected with the salt proportions of solution R3C5. Thus, the maximum yield of tops was produced during the later period of growth (series B) in a medium having a lower osmotic proportion of monopotassium phosphate, a much higher proportion of calcium nitrate, and a much lower one of magnesium sulphate than the solution which produced the highest yield of tops during the early growth period (series A).

A comparative study of the diagrams of the two series thus brings out the fact that there is no similarity between the two series with respect to the distribution of the areas of high and of low dry-weight yields of tops. This is a clear indication that the response of the plants to the osmotic proportions of the salts in the solutions here employed is markedly different for the two different stages of development. Furthermore, a comparison of the total ranges of the average relative yields of the two series clearly shows that the buckwheat plants here employed respond just as readily to the variations in the proportions of the salts in the different solutions during the later period of development (series B) as they do during the early stages of growth (series A). The variation in the average relative yield values for tops in series A extends from 0.60 to 1.34, showing a total range of 0.74 from the lowest to the highest value. In series B the corresponding total range from the lowest to the highest is 0.80, extending from 1.00 to 1.80. The variations in the average yield values as given for series B must, of course, be taken as the results of the approximate differences in the growth rates of the cultures, during the time period of this series, in response to the differences in the salt proportions in the various solutions. Since, however, the cultures were carefully selected and, so far as could be judged, were

all nearly alike when the series was begun, the average yield values may be considered to approximate very closely the values which would have obtained if all the plants had been exactly alike at the beginning of the second 4-week growth period.

(*b*) COMPARISON OF THE RANGES OF THE ION RATIO VALUES FOR HIGH AND FOR LOW YIELDS OF TOPS.—The ion ratio values of the 3-salt solutions here employed have been discussed in detail in previous publications (*6*, *7*) in connection with the study of the growth of young wheat and buckwheat plants in these solutions, in an endeavor to determine the relation of these ratio values to the physiological properties of the various salt combinations as they affect the growth of the plants. These ion ratio values have also been considered by McCall (*3*) in his study of young wheat plants in sand cultures.

In Table III are presented the cation ratio values of the nine cultures of each of the two series here considered, giving the highest dry weights of tops and of the nine cultures giving the lowest corresponding weights. The cultures are in every case arranged in the descending order of the values of the magnesium to calcium ratio. These cultures are the ones included in the areas of high and of low yields outlined on the triangular diagrams of figure 1. The table is divided into two vertical sections; the first section gives the culture numbers and the three cation ratio values of each of the nine cultures which produced high yields and the nine which gave low yields of tops in series A and also the total range in the magnitude of these ratio values. The second section presents the corresponding data for series B. At the bottom of the table are given the maximum and minimum ratio values and the total ranges of these for the entire series. The ratio values of the culture giving the highest yield in each series are indicated in bold-face type, while those of the culture giving the lowest dry weight appear in italics.

It will be observed that the ion-ratio values characterizing the cultures in each of the two series giving high and low yields of tops are limited to certain ranges of these ratio values, which are less extensive than the total ranges for the entire series.

From a comparison of the ratio values for the group of cultures producing high yields in series A with those of the corresponding group in series B, it may be seen that there is substantial agreement between the two series with respect to the ranges of the magnesium to potassium ratio values. This group of cultures in each of the two series is characterized by a relatively low range of values for this ratio, the range being 3.93 for series A and 3.71 for series B. There is, however, no such agreement between the two series with respect to the range of the values for the magnesium to calcium and calcium to potassium ratios. The range of the former is high (12.69) in series A and relatively low (5.53) in series B, while the range of the latter is relatively low (1.30) in series A and high (5.22) in series B.

TABLE III.—*Comparison of ion ratio values of cultures producing high and low yields (best nine and poorest nine cultures) of buckwheat tops during the early period of growth (series A) with corresponding data for cultures grown during the late period of growth (series B)*

Series A (early growth period.)				Series B (late growth period).			
Culture No.	Magnesium: calcium.	Magnesium: potassium.	Calcium: potassium.	Culture No.	Magnesium: Calcium.	Magnesium: potassium.	Calcium: potassium.
High yields:				High yields:			
R2C1	13.46	4.86	0.36	R2C2	5.77	4.17	0.72
R3C1	11.55	2.87	.24	R3C2	4.81	2.32	.84
R4C1	9.61	1.74	.18	R4C3	1.92	1.04	.54
R5C1	7.70	1.11	.14	R3C4	1.44	1.39	.96
R2C2	5.77	4.17	.72	R4C4	.96	.69	.72
R3C2	4.81	2.32	.84	R3C5	**.77**	**.93**	**1.20**
R4C2	**3.85**	**1.39**	**.36**	R1C7	.55	2.78	5.04
R2C4	1.92	2.77	1.44	R3C6	.32	.46	1.44
R3C5	.77	.93	1.20	R1C8	.24	1.39	5.76
Range	12.69	3.93	1.30	Range	5.53	3.71	5.22
Low yields:				Low yields:			
R7C1	3.85	.40	.10	R1C1	*15.40*	*11.10*	*.76*
R1C4	*2.40*	*6.95*	*2.88*	R2C1	13.46	4.86	.36
R8C1	1.92	.18	.09	R3C1	11.55	2.76	.24
R3C4	1.44	1.39	.96	R4C1	9.61	1.74	.18
R5C3	1.28	.56	.43	R5C1	7.70	1.11	.14
R7C2	.96	.20	.20	R6C1	5.77	.69	.12
R6C3	.64	.23	.36	R1C3	3.85	8.34	2.16
R4C5	.38	.35	.90	R1C6	.96	4.17	4.32
R3C6	.32	.46	1.44	R4C5	.38	.35	.90
Range	3.53	6.77	2.79	Range	15.02	10.75	4.20
Entire series:							
Maximum	15.40	11.10	5.76				
Minimum	.24	.18	.09				
Range	15.16	10.92	5.67				

The cultures R4C2 and R3C5, which produced the highest yields of tops in series A and series B, respectively, agree in showing relatively low values for all three cation ratios, although there is considerable difference between the corresponding ratio values for the two cultures. The values of the three ratios magnesium to calcium, magnesium to potassium, and calcium to potassium for the culture (R4C2) producing the maximum yield of tops in series A are 3.85, 1.39, and 0.36, respectively, while the corresponding ratio values for the culture (R3C5) which produced the highest yield in series B are 0.77, 0.93, and 1.20, respectively.

On comparing now the ranges in the ratio values as given in Table III for low yields it will be observed that there is no agreement between the groups of cultures producing low dry weights in the two different series, with respect to the ranges in the magnitudes of any two corresponding ratios. Thus, the group of cultures of series A giving low yields of tops is characterized by a relatively low range of values for the ratio mag-

nesium to calcium, and an intermediate range for the ratios magnesium to potassium and calcium to potassium, while the group of cultures which produced low yields in series B shows a wide range in the values for each of the three ratios.

The individual culture R1C4 giving the lowest dry weight of tops in series A shows a low magnesium to calcium ratio value of 2.40 and intermediate values of 6.95 and 2.88 for the ratios magnesium to potassium and calcium to potassium, respectively. Culture R1C1, which gave the lowest yield of tops in series B, is characterized by the maximum values of the ratios magnesium to calcium and magnesium to potassium, and by a low value of the ratio calcium to potassium. The values of these three ratios are 15.40, 11.10, and 0.76, respectively. Thus, there is no agreement between any two corresponding ratio values characterizing the individual cultures which produced the minimum yields in their respective series.

From the above considerations it is at once clear that these ion ratio values and the dry-weight yields of tops are not at all related in the same way in the two series representing the two physiological stages of development in the active growth period of the plants. This is still further emphasized by the marked differences in the atomic proportions characterizing the solutions producing the highest and lowest yields in the two series. Thus, for example, the highest yield in series A occurred with the solution R4C2, having the ratio values as follows: magnesium to calcium, 3.85; magnesium to potassium, 1.39; and calcium to potassium, 0.36. This indicates that the best solution for tops in this series contains 1.39 atoms of magnesium and 0.36 atoms of calcium for each single atom of potassium (by assuming, of course, that the number of atoms of an element present in a given mass of it is proportional to the number of gram-atoms contained in the mass). The solution (R3C5) which produced the highest yield in series B possessed 0.93 atoms of magnesium and 1.20 atoms of calcium for each atom of potassium. The difference between the atomic proportions characterizing the solutions in the two series which produced the poorest growth of tops is even more pronounced than is that between the atomic proportions characterizing the solutions which produced the highest yields. The solution (R1C4) giving the lowest yield of tops in series A had the atomic proportions magnesium, 6.95; calcium, 2.88; and potassium, 1.00, while the solution (R1C1) giving the lowest yield of tops in series B, possessed the proportions magnesium, 11.10; calcium, 0.76; and potassium, 1.00. It is thus obvious that the atomic proportions characterizing the solutions producing the best and also the poorest yields of tops vary markedly with the different growth periods here considered.

(2) DRY WEIGHTS OF ROOTS

The average relative dry weights of roots are given in Table II in connection with the corresponding data for tops. These relative root yields

have been plotted on triangular diagrams like those of figure 1, which graphically represent the relative yields of tops. The effect of the differences in the developmental stages of the plants upon the positions and ranges of the areas of high and low yields of roots will be compared by referring to the triangular diagrams of figure 2, in which the lower diagram represents the average yields of roots from series A, and the upper one represents the yields from series B.

(a) COMPARISON OF THE EFFECTS OF THE VARIOUS OSMOTIC SALT PROPORTIONS FOR THE TWO PHYSIOLOGICAL GROWTH PERIODS—CONSIDERATION OF THE RELATIVE DRY WEIGHTS OF ROOTS.—A comparison of the diagram of series A with that of series B shows the main areas of low yields to lie on opposite margins of the diagram. There is no overlapping of the areas of low yields. The cultures (R1C4 and R1C1) producing lowest yields of roots in series A and B, respectively, are the same ones which produced also the minimum yields of tops in the corresponding series. The osmotic proportions of the three salts characterizing these two solutions are therefore the same for both tops and roots.

The main areas of high yields on the two diagrams occupy central regions lying chiefly on opposite sides of the vertical axes of the diagrams. The main high area of series A extends to the left and includes the three marginal cultures R4C1, R5C1, and R6C1, while the main area of high yields on the diagram for series B extends to the right margin at culture R3C6. There is a certain amount of overlapping of the areas of high yields, but only a single culture is shown which is common to areas of high yields on the two diagrams. This culture (R3C5), which marks the lower limit of the range of high yields in series A, produced the maximum yield in series B. The maximum yield of roots in series A and that in series B occurred with the cultures R4C2 and R3C5, respectively. These two cultures have already been shown on the diagrams of figure 1 as producing the highest dry weights of tops in their respective series. Thus the osmotic proportions characterizing these two solutions, like those characterizing the solutions producing the lowest yields in the two series are the same for both tops and roots.

It is obvious that there is as little similarity, with respect to the distribution of the areas of high and low yields, between the two diagrams representing the yields of roots (fig. 2) as there is between those representing the yields of tops (fig. 1). From these considerations it is clear that the various osmotic proportions of the three salts in the solutions here employed affect the growth of roots as differently during the two developmental periods as they do the growth of tops.

A comparison of the two diagrams of figure 2 with the corresponding ones of figure 1, representing the relative yields of tops, brings out some very striking correlations between the growth of tops and that of roots. As has already been pointed out, the cultures in each of the two series which produced the highest yields of tops, gave also the maximum root

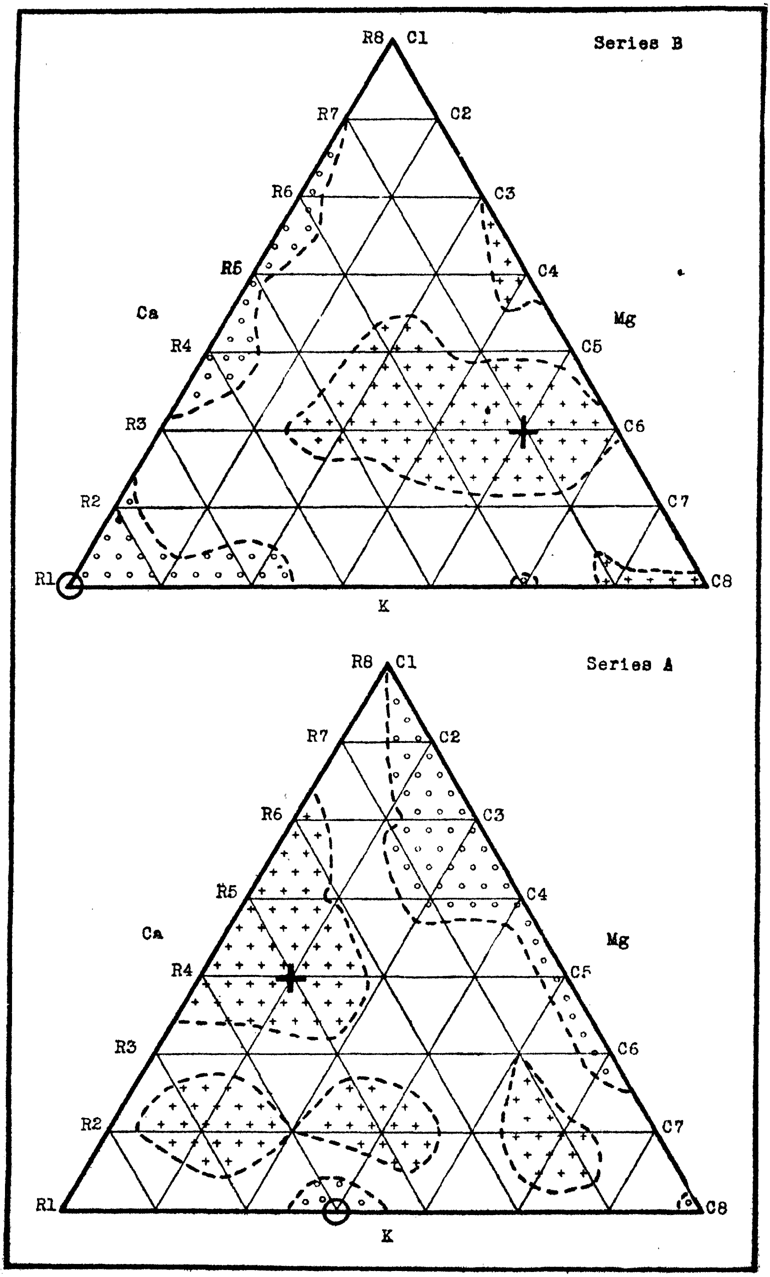

FIG. 2.—Diagrams showing relative yields of buckwheat roots. Areas of high yields are indicated by small crosses, those of low yields by small circles. Cultures giving the highest and lowest yields are marked by larger crosses and larger circles, respectively.

yields, while the cultures which yielded the lowest dry weights of tops gave also minimum root yields. It will further be observed that the diagrams graphically representing the dry weights of tops (fig. 1) and the corresponding ones representing the relative yields of roots, show a very pronounced similarity with respect to the positions and ranges which the areas of high and low yields occupy on the diagrams.

(b) COMPARISON OF THE RANGES OF THE ION RATIO VALUES FOR HIGH AND FOR LOW YIELDS OF ROOTS.—The cation ratio values of the nine cultures producing the best yields of roots, and also the group of nine cultures giving the poorest yields, and the ranges of these values for each of the two series here considered, are presented in Table IV, which conforms in every respect to Table III. As in the case of top yields, the ion ratio values for the best nine and the poorest nine cultures in each of the two series are limited to certain ranges of these ratio values, which are always less extensive than the corresponding total ranges for the entire series.

TABLE IV.—*Comparison of ion ratio values of cultures producing high and low yields (best nine and poorest nine cultures) of buckwheat roots during the early period of growth (series A) with corresponding data for cultures grown during the late growth period (series B)*

Series A (early growth period).				Series B (late growth period).			
Culture No.	Magnesium : calcium.	Magnesium : potassium.	Calcium : potassium.	Culture No.	Magnesium : calcium.	Magnesium : potassium.	Calcium : potassium.
High yields:				High yields:			
R4C1	9.61	1.74	0.18	R3C3	2.56	1.85	0.72
R5C1	7.70	1.11	.14	R4C3	1.92	1.04	.54
R2C2	5.77	4.17	.72	R3C4	1.44	1.39	.96
R6C1	5.77	.69	.12	R3C5	.77	.93	1.20
R4C2	3.85	1.39	.36	R6C3	.64	.23	.36
R2C3	3.21	3.47	1.08	R1C7	.55	2.78	5.04
R2C4	1.92	2.77	1.44	R5C4	.48	.28	.58
R3C5	.77	.93	1.20	R3C6	.32	.46	1.44
R2C6	.64	1.39	2.16	R1C8	.24	1.39	5.76
Range	8.97	3.48	1.32	Range	2.32	2.55	5.40
Low yields:				Low yields:			
R1C4	*2.40*	*6.95*	*2.88*	R1C1	*15.40*	*11.10*	*.72*
R8C1	1.92	.18	.09	R2C1	13.46	4.86	.36
R5C3	1.28	.56	.43	R4C1	9.61	1.74	.18
R7C2	.96	.20	.20	R5C1	7.70	1.11	.14
R6C3	.64	.23	.36	R1C2	6.74	9.72	1.44
R5C4	.48	.28	.58	R6C1	5.77	.69	.12
R4C5	.38	.35	.90	R1C3	3.85	8.34	2.16
R1C8	.24	1.39	5.76	R7C1	3.85	.40	.10
R3C6	.32	.46	1.44	R1C6	.96	4.17	4.32
Range	2.16	6.77	5.67	Range	14.44	10.70	4.22
Entire series:							
Maximum	15.40	11.10	5.76				
Minimum	.24	.18	.09				
Range	15.16	10.92	5.67				

From Table IV it will be observed that the iron-ratio ranges for the cultures giving high or low yields of roots vary as markedly and in the same manner with the two different developmental growth periods (series A and B) as they do for the corresponding yields of tops. Thus, the cultures giving high root yields in series A show a wide range (8.97) in the magnesium to calcium ratio values and low ranges (3.48 and 1.32, respectively) in the values of the magnesium to potassium and calcium to potassium ratios, while the cultures of series B giving corresponding yields possess low ranges (2.32 and 2.55, respectively) for the magnesium to calcium and the magnesium to potassium ratio values, and a wide range (5.40) in the values of the calcium to potassium ratio. The group of cultures which produced low root yields in series A shows a low range (2.16) of values for the magnesium to calcium ratio, an intermediate range (6.77) for the magnesium to potassium ratio, and a wide range (5.67) for the calcium to potassium ratio. In series B the group of cultures giving low yields of roots is characterized by wide ranges in the values of all three ratios. These values are 14.44, 10.70, and 4.22, for the ratios magnesium to calcium, magnesium to potassium, and calcium to potassium, respectively.

A comparison of the data in Table III with those in Table IV brings out the fact that nearly all the ratio ranges for the cultures producing either high or low yields of tops show substantial agreements with the corresponding ranges for the cultures in the same series giving high or low root yields. It thus appears that the relation of high or low root yields to the proportions of the chemical ions is, in a general way, similar to the relation of the high and low yields of tops to these ion-ratio values. This follows, of course, from the general similarity of the positions and ranges of the areas of high and low yields on the corresponding triangular diagrams of figures 1 and 2. Since in the same series the maximum yields of tops and of roots occurred with the same culture, as did also the minimum yield of tops and of roots, there is but one set of ratio values for the two kinds of maximum yields and one set for the two kinds of minimum yields for each of the two series.

(3) DRY WEIGHTS OF SEEDS

The absolute and relative dry weights of seeds obtained from the cultures of series B are presented in Table I, in connection with the corresponding data for tops and for roots. The absolute dry weight values represent, in each case, the averages from two series. The relative values were obtained, as were also the relative yields of tops and of roots, by dividing the average absolute dry weight value for each culture by the corresponding value for culture R1C1. The ratios of top yields to the yields of seeds are given in the last column of Table I. These ratio values represent the yields of tops expressed in terms of the corresponding yields of seeds considered as unity.

The average relative yields of seeds are represented graphically on the triangular diagram of figure 3, in the same manner as are the yields of tops and of roots on the diagrams of figures 1 and 2, respectively. The range of the average relative yields of seeds extends from 0.35 to 2.62. On the diagram representing these yields the main area of high dry weights (2.14–2.62) occupies a central region at the base of the triangle, extending upward to the right margin at culture R5C4. A small outlying high area is also indicated about culture R2C7. The highest yield of seeds occurred

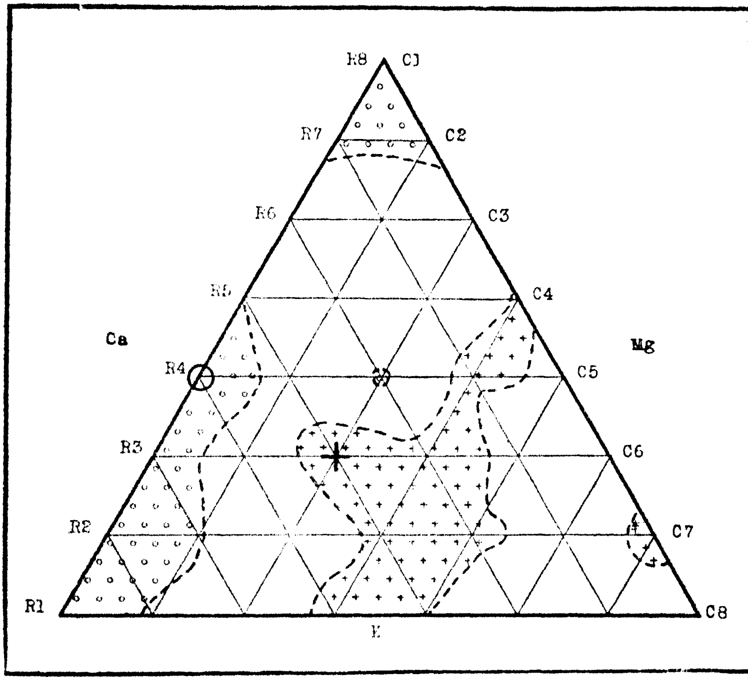

FIG. 3.—Diagram showing relative yields of buckwheat seeds. Areas of high yields are indicated by small crosses, those of low yields by small circles. The culture giving the highest yield is marked by a larger cross, that giving the lowest yield by a larger circle.

with culture R3C3, which produced a medium yield of tops. The main area of low yields (0.35–1.31) extends along the left margin to the base of the triangle, and a smaller low area is shown at the apex and another at culture R4C3 at the center of the diagram. The lowest yield of seeds was produced by the plants of culture R4C1. A comparison of the diagram of figure 3 with the upper diagram of figure 1, representing the yields of tops, shows that there is little correlation between high top yields and the production of large yields of seeds. Only two cultures, R3C4 and R4C4, which produced high top yields are included in the areas of high yields of seeds, and these two cultures mark the lower limit in the range of high yields of tops. On the other hand, the main area of

low yields of seeds, extending along the left margin of the diagram, has a corresponding area of low top yields. It is to be noted, however, that two cultures, R2C2 and R4C3, which produced high dry weights of tops, lie within the areas of low yields of seeds.

It thus appears that the yields of tops and of seeds do not vary in the same way with reference to the variations in the proportions of the three salts in the solutions. It is entirely possible, of course, that this lack of correlation between the growth of tops and the production of seed is related to other factors than the physiological properties of the media in which the plants were grown. Several of the more important of these factors may here be mentioned. The plants of a series of cultures like those here employed must vary greatly in the degree of productiveness, which is certainly much higher in some plants than in others, even when grown under exactly similar conditions with respect to the medium and the aerial surroundings. This is apparently an hereditary quality and one certainly not easily to be controlled in experiments of this character. Furthermore, these series of cultures were conducted during a season of the year when insect pollination was not possible, and pollination by artificial means was perhaps imperfectly accomplished, or it may have been less perfectly accomplished with some of the cultures than with others. Either one or both of these factors may have exerted an influence upon the manner in which the yields of seeds varied throughout the series. Whatever influence these factors may have had upon seed production, it is certain that an abundance of seed was produced by nearly all the cultures, as will be seen from an inspection of the last three columns of Table I, giving the numerical data of seed yields. From the last column of this table showing the ratios of the yields of tops to the yields of seeds, it will be observed that the ratio values for eight cultures lie between 2.0 and 3.0, while the ratios for 26 cultures have values between 1.0 and 2.0. The average of these ratio values for the entire series is 1.94. This indicates that the average yield of tops for the entire series is less than double the corresponding yield of seeds. Nearly all the seeds obtained from these cultures were large, fairly uniform in size, and well filled. Very few small or imperfectly formed seeds were present.

II.—TRANSPIRATION AND WATER REQUIREMENTS

As previously stated, the total amount of water lost by transpiration during the growth period was determined for each culture by summing the losses recorded for the partial periods between each two successive changes of solutions. From the total water loss for each culture considered in connection with the dry weights of tops, of roots, and of seeds, have been derived the ratios representing the amount of water lost by transpiration for each single gram of dry plant substance produced. These ratios of transpiration to yields represent the water requirements of the plants. Table V presents the data of transpiration for the series

(A and B) of the two developmental periods here considered, and also the data of water requirements for the cultures of the two series. The various measurements for each culture are expressed in terms of the corresponding measurement for culture R1C1 in the respective columns. The actual value for this culture is given in each case in parentheses just below the relative value, 1.00. In columns 2 and 3 are given the relative amounts of water loss for the cultures of series A and B, respectively. Then follow two columns presenting the relative water requirements of tops and roots for series A. The last three columns give the relative water requirements for tops, roots, and seeds, for series B.

TABLE V.—*Data of transpiration and water requirement: series A, grown to the flowering stage; series B, grown from the flowering stage to maturity in 3-salt solutions*

Culture No.	Transpiration.		Water requirements.				
	Series A.	Series B.	Series A.		Series B.		
			Tops.	Roots.	Tops.	Roots.	Seeds.
R1C1	1.00 (205)	1.00 (763)	1.00 (389)	1.00 (6710)	1.00 (478)	1.00 (5990)	1.00 (1282)
R1C2	.84	1.12	1.01	1.08	.91	.87	.75
R1C3	.97	1.10	.99	.90	1.05	.97	.66
R1C4	.67	1.12	1.17	.95	.86	.71	.73
R1C5	.95	1.19	1.06	.88	.88	.78	.65
R1C6	1.06	1.10	1.06	.89	.96	.77	.62
R1C7	1.08	1.40	1.08	.92	1.00	.78	.88
R1C8	.96	1.29	1.13	1.10	.96	.85	.67
R2C1	1.03	1.03	1.00	.90	.99	.93	1.12
R2C2	1.18	1.17	1.05	.81	.83	.66	.81
R2C3	.96	1.43	1.01	.79	1.19	.98	.99
R2C4	1.12	1.33	.95	.81	1.10	.99	.52
R2C5	.91	1.34	.98	.84	1.13	.86	.60
R2C6	1.06	1.29	1.05	.82	1.14	.84	.71
R2C7	1.06	1.26	1.15	.93	.95	.82	.50
R3C1	.99	1.15	.92	.92	.83	.69	.71
R3C2	.98	1.23	.95	.86	.92	.80	.95
R3C3	1.01	1.36	.84	.73	1.08	.65	.58
R3C4	.91	1.37	1.11	.85	.99	.68	.89
R3C5	1.26	1.46	1.20	1.03	.67	.44	.61
R3C6	.81	1.36	1.13	1.10	.85	.67	.64
R4C1	1.05	.76	1.03	.73	.66	.70	1.63
R4C2	1.07	1.36	.99	.84	1.09	.90	.72
R4C3	.98	1.25	1.03	.83	.76	.73	1.23
R4C4	1.03	1.31	1.08	.85	.98	.75	.84
R4C5	.87	1.17	1.27	1.15	1.04	.93	.75
R5C1	1.07	.83	1.02	.82	.69	.61	2.30
R5C2	1.04	1.34	1.17	.87	1.06	.85	.67
R5C3	.91	1.18	1.19	1.13	1.07	.74	.73
R5C4	.95	1.34	1.15	1.23	1.12	.82	.79
R6C1	1.00	1.13	1.00	.75	.97	.83	.72
R6C2	.96	1.22	1.13	.99	.96	.70	.74
R6C3	.84	1.36	1.24	.97	1.11	.87	.86
R7C1	.88	1.16	1.19	.89	.90	.85	1.26
R7C2	.75	1.17	1.21	.87	.92	.90	2.34
R8C1	1.03	1.08	1.29	1.13	.75	.67	.70
K	.93	1.36	1.11	.98	1.01	.58	1.71
T	1.15	1.25	1.14	.97	1.03	.66	1.25

In order to facilitate comparisons, the average data of Table V were plotted on triangular diagrams in the same manner as were the dry-weight yields. The corresponding diagrams of the two series thus obtained were then compared and a comparison was also made with the corresponding yield diagrams (fig. 1, 2). These diagrams graphically representing the average data of Table V are not here given, but the chief points brought out by these comparisons are presented below:

A.—Relation of Transpiration to Yields

A comparison of the transpiration diagrams of series A with the corresponding yield diagrams shows the main areas indicating high transpiration rates, and also those denoting low rates, to occupy positions corresponding fairly well, though not absolutely, with the areas of high and of low yields, respectively, both of tops and of roots. In series B there is equally good agreement between transpiration and yields of tops and of roots, both for the areas of high yields and for those of low yields. It is thus clear that, in general, high transpiration corresponds to high yields of tops and of roots for each of the two developmental periods represented by series A and series B. It is to be emphasized, however, that there is no agreement between the areas of high and low values on the corresponding transpiration diagrams of the two series. This is a clear indication that the relation between the various salt proportions and transpiration is as widely different with respect to the two periods of development here considered as is the relation between these salt proportions and the yields, either of tops or of roots.

B.—Relation of Water Requirements to Yields

A study of the five triangular diagrams representing the water requirement data in Table V brings out several interesting relations. Thus, in series A, a definite relation is shown between the water requirements of tops and top yields and also between the water requirements of roots and root yields. On the diagram representing water requirements of tops, in this series, the areas of high values correspond to the areas of low values on the diagram of top yields, while the regions of low water requirement values correspond to the areas of high top yields. These relations are shown to hold equally well between the water requirements of roots and root yields. In series B the diagrams representing the water requirements of tops and of roots, when compared with the corresponding yield diagrams, show a marked tendency toward the same relations. This is particularly true with respect to the relations between low water requirements and high yields, both for tops and for roots, although the agreements are not so exact as they are in series A. There is, however, no detailed agreement between the areas of low yields and those of high water requirements.

A comparison of the water requirement diagram for seeds with the corresponding yield diagram indicates a very close agreement between the areas representing high water requirement and those of low yields. The areas of low water requirement values correspond also, in a general way, with the areas of high yields.

From the above observations it is clear that a fairly definite relation exists between the dry-weight yields and water requirements for each of the two series of buckwheat plants here considered. This relation may briefly be stated as follows: In general, high yields of tops, roots, and seeds correspond to low water requirements, and low yields correspond to high water requirements. It is thus to be expected that favorable conditions for the growth of these plants are associated with relatively low water requirements, while unfavorable conditions for growth will demand a relatively larger amount of water to produce 1 gm. of dry plant substance.

A comparison of the water-requirement diagrams of series A with the corresponding diagrams of series B brings out the fact that there is no similarity between the diagrams of the two series representing the two developmental growth periods with respect to the positions and distribution of the areas of high and low water requirement values. Thus there is a marked difference in the manner in which the water-requirement values of the two series vary with respect to the variations in the salt proportions of the solutions employed.

From the preceding considerations of the various plant measurements it is at once clear that the relation of the growth rate of the buckwheat plants to the proportions of the three salts in the solutions here employed is markedly different for the early and late developmental periods represented by series A and series B, whether this relation is judged by the criterion of dry weights of tops or of roots, by that of transpiration, or by that of water requirements of tops or of roots.

It is to be emphasized, of course, that the changes in the physiological requirements of these plants, with respect to the salt proportions, may be a gradual process extending over a comparatively long time period. Such a change might even begin soon after germination of the seed and continue during the entire active growth period of the plants. If, therefore, the entire growth period should be divided into a larger number of partial periods than the two which have here been considered, and the best physiological balance of salt proportions determined for each, these partial periods might possibly call for other salt proportions for the production of maximum yields than the ones which gave the highest yields during the first or during the last 4-week period of these tests. On the other hand, the change in the salt requirements of the plants may take place comparatively rapidly with the marked changes which occur within the plants during the period of blossoming, when the vegetative processes become less active and the reproductive and seed-forming processes begin.

SUMMARY

The preceding pages present a comparative study of the salt requirements for young and for mature buckwheat plants grown in nutrient solutions having the same initial total concentration value of 1.75 atmospheres, but differing in the proportions of the component salts. This series of solutions comprised 36 different sets of salt proportions of the three salts, potassium phosphate (KH_2PO_4), calcium nitrate[$Ca(NO_3)_2$], and magnesium sulphate ($MgSO_4$).

The results obtained from a series of cultures grown in these solutions, from the flowering stage to the maturity of the plants, were compared with those obtained from a similar series previously carried out with the same solutions but conducted only to the flowering stage. The main results of this comparative study may be summarized briefly as follows:

(1) The highest yield of buckwheat tops and of roots obtained in a period of four weeks directly following germination was produced by a solution characterized by the following salt proportions: Potassium phosphate, 0.0144 m; calcium nitrate, 0.0052 m; magnesium sulphate, 0.0200 m. The buckwheat plants grown during the second four-week period (including the period of seed production and ripening) in the same series of 3-salt solutions as were the plants harvested at the end of the first four-week period produced their highest yield of tops and of roots in a solution having the salt proportions of potassium phosphate, 0.0108 m; calcium nitrate, 0.0130 m; magnesium sulphate, 0.0100 m. Thus, the maximum yield was produced during the later stage of development (series B) in a medium having a lower osmotic proportion of potassium phosphate, a much higher proportion of calcium nitrate, and a much lower one of magnesium sulphate than had the medium which produced the highest yield during the early growth period (series A).

(2) The buckwheat plants respond just as readily to the variations in the osmotic proportions of the salts in the different solutions during the later period of development as they do during the early stage of growth, but this response is markedly different for the two different stages of development.

(3) The values of the cation atomic ratios magnesium to calcium, magnesium to potassium, and calcium to potassium, characterizing the solutions which produced the highest yields, and also those which gave the lowest yields, differ markedly with the two different developmental stages of the plants.

(4) The amounts of transpirational water loss during each of the two different periods of development, indicate, in a general way, the yields. High transpiration corresponds to high yields of tops, and low transpiration to low yields.

(5) For each of the two developmental periods of growth considered, low water requirement is, in general, associated with high yields of tops and of roots, and high water requirement with low yields.

(6) There is no definite correlation between the yields of tops and of seeds, such as there is between the yields of tops and of roots.

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COMPOSITION AND DIGESTIBILITY OF SUDAN-GRASS HAY

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INTRODUCTION

The introduction of Sudan grass (*Andropogon sorghum* var.) into the United States took place less than nine years ago (1909), but since then this crop has become widely known, and its popularity is rapidly increasing. Sudan grass, being an annual, does not make a good pasture plant, but gives excellent results as a hay or soiling crop; it might also be successfully made into silage if mixed with a legume.

RÉSUMÉ OF PREVIOUS WORK

A considerable amount of work has been done on the production of Sudan grass; and, though the yields of hay obtained varied considerably, they were as a rule satisfactory (Table I).

TABLE I.—*Average yields of Sudan-grass hay* (*5*)[a]

State Experiment Station.	Dry hay per acre.	State Experiment Station.	Dry hay per acre.
	Tons.		*Tons.*
Virginia	3.4	Texas	3.9
Tennessee	2.6	Oklahoma	2.9
Mississippi	5.5	Ohio (*8*)	4.3
Louisiana	3.3	Kansas (*7*)	3.1
Georgia	3.6		
Arkansas	1.1	Average	3.4

The average yields of Sudan-grass hay, as stated in Table I, have not all been calculated by the same method, but the results show that as a rule a yield of 3 to 4 tons of field cured hay per acre can be expected.

The material available to show the composition of Sudan-grass hay is limited, but a compilation of the published analyses is included here. There is a wide variation in the moisture contents of hays, due to a

[a] Reference is made by number (italic) to "Literature cited," p. 185.

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considerable extent to the lack of uniformity in the conditions under which curing takes place; so in Table II the various constituents are expressed as percentages of the total dry matter present in the samples of hay analyzed.

TABLE II.—*Composition of the dry matter of Sudan-grass hay*

Constituent.	Maryland (6).	Virginia (4).	Texas (9).	Oklahoma (3).	Average.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Total dry matter	90. 12	96. 49	....	92. 80	93. 14
Protein	6. 57	4. 83	12. 42	8. 56	8. 10
Nitrogen-free extract	51. 99	51. 09	45. 56	48. 98	49. 41
Crude fiber	34. 83	36. 92	29. 93	34. 01	33. 92
Ether extract	1. 88	1. 32	1. 92	2. 42	1. 89
Ash	4. 74	5. 85	10. 16	6. 03	6. 70

The analyses of Sudan-grass hay that have been reported are fairly uniform in all their constituents except protein and ash, which show rather wide variations, due perhaps to the conditions under which the crops were grown and the stage of growth at the time of cutting.

It is generally understood that the majority of crops alter materially in composition as ripening progresses. This change is due not only to the increase in the amount of dry matter and the decrease in the amount of water but also to a variation in the relative proportions of the individual constituents of the dry matter. These changes usually go on until the crop is practically ripe, but that this is not so in the later stages of ripening in the case of Sudan grass has been shown by Piper (Table III).

TABLE III.—*Composition of dry matter of Sudan-grass hay (4) made at various stages of ripeness*

Stage of cutting.	Before heading.	Heads appearing.	Beginning to bloom.	In full bloom.	Seeds fully mature.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Protein	8. 08	6. 28	5. 34	4. 83	4. 38
Nitrogen-free extract	51. 23	53. 41	53. 76	51. 09	55. 85
Crude fiber	32. 00	33. 11	34. 42	36. 92	36. 02
Ether extract	1. 79	1. 44	1. 27	1. 32	1. 55
Ash	6. 89	5. 75	5. 20	5. 85	5. 85

As would be expected, there is a decrease in the protein and a slight increase in the crude-fiber content. These changes are marked in the case of the protein, but the other constituents are fairly constant. The significance of this is that from the time Sudan grass heads out until it is fully ripe there is very little change in the fiber content of the dry matter, and consequently the time of cutting can be delayed without much risk of the hay becoming too coarse. This suggests a distinct

advantage if the haying season is wet; the cutting of the Sudan grass may advantageously be postponed for a week or 10 days if there is a prospect of improvement in the weather.

In spite of the fact that Sudan grass is now grown in quite an extensive territory, it has been fed but little experimentally. Large amounts of Sudan hay are consumed annually, yet only in one or two cases have accurate records been kept of the results it produced.

So far only one digestion trial has been conducted with Sudan-grass hay. This work consisted of a 5-day test period with a 2-year-old bull, and the results of it are given in Table IV.

TABLE IV.—*Digestibility of Sudan-grass hay* (*6*)

Constituent.	Digestion coefficient.
	Per cent.
Dry matter	60. 6
Crude protein	35. 4
Nitrogen-free extract	63. 3
Crude fiber	67. 1
Ether extract	41. 2

The digestion coefficients for Sudan-grass hay obtained at the Maryland Experiment Station compare well with those for other nonleguminous roughages (*6*).

At the Kansas Experiment Station Sudan-grass hay was compared with alfalfa hay as a roughage for dairy cows. Two lots of three cows each were used. There were two 30-day test periods. In the first period Lot I received alfalfa hay and Lot II Sudan-grass hay, while in the second test period the roughages for the two lots were reversed (Table V).

TABLE V.—*Sudan-grass hay v. alfalfa hay for milk production*

	Roughage.		Gain due to alfalfa.
	Sudan grass.	Alfalfa.	
	Pounds.	*Pounds.*	*Pounds.*
Milk produced	4, 022	4, 112	90
Fat produced	168	178	10
Average body weight	1, 053	1, 077	24

This shows a difference in production of 0.5 pound of milk per head per day in favor of the alfalfa hay. This is not a large difference, but if the experiment had been run for another 30-day period so as to facilitate the elimination of the decrease in production due to advance in lactation, there is little doubt that the Sudan-grass hay would have shown up even less favorably. The fact that the cows increased in weight when receiving the alfalfa is significant (*6*).

The Kansas records (7) also show that when the herd of milking cows was turned from a native pasture on to a Sudan pasture the average daily production of milk was increased 3.2 pounds per head, even though Sudan grass is not a first-class pasture plant. In addition, they also found that for wintering work horses and mules and young beef cattle Sudan-grass hay was of considerably less value than alfalfa hay.

EXPERIMENTAL WORK

The Sudan grass used in the work reported in this paper was grown on the College dairy farm. During the two years in which this crop has been grown there it has given good results as a soiling crop, the average yield being 11 tons of green feed per acre for one cutting. In 1916 a small amount of second growth was made into hay. Sudan grass seems to be palatable and much relished by the stock, and good results have been obtained in the feeding of both the soiling and the hay.

In 1915 analyses were made of the crop at various stages of growth. The samples were all taken from one small plot in the center of the area grown for soiling and the results of the analyses are expressed as percentages of the total dry matter present.

TABLE VI.—*Composition of dry matter of Sudan grass at various stages of growth*

Stage of growth.	Before heading.	Headed out.	Full bloom.	Half ripe.	Ripe.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Total dry matter	20.80	20.96	25.74	30.08	31.92
Protein	8.80	9.78	6.57	5.02	4.29
Nitrogen-free extract	48.12	46.04	50.19	53.32	53.73
Crude fiber	32.98	35.50	32.36	32.98	33.83
Ether extract	2.31	2.62	3.53	2.10	1.66
Ash	7.79	6.06	7.35	6.58	6.49

As the moisture decreases and the dry matter content increases in the later stages of growth of Sudan grass, a few minor changes take place in the relative proportions of the individual constituents of the dry matter. In the earlier stages of ripening the protein seems to increase, while it decreases in the later stages. The changes in the fat content are very similar to those of the protein content, but lag behind them. The changes in the proportions of nitrogen-free extract and ash are in the opposite direction to those of the protein and ether extract. Peculiarly, the relative proportion of the crude fiber to the other constituents of the dry matter appears to be greater when the plants have headed out than when the crop is ripe. The difference is not great, however, and can probably be explained by the fact that the seed, of which the yield is quite heavy, is very low in crude fiber. It has been found at the Maryland Station (6) that cleaned Sudan-grass seed contains only 1.19

per cent of crude fiber. To consider the changes broadly, it is evident that from the time the crop heads out until it is ripe no very marked alterations take place in the relative proportions of the various constituents of the dry matter present, and consequently Sudan grass does not materially deteriorate in feeding value on ripening.

The hay used in the digestion trial was from a plot yielding 2.94 tons of field-cured hay per acre at one cutting. It was cut on August 5, 1916, when in full bloom and was harvested in good condition. It was kept in the mow till used for the digestion trial in December, 1916.

The animals used were two three-quarter blood Guernsey heifers about a year and a half old and averaging 600 pounds in live weight. These animals were of 75 per cent the same breeding, being sired by Rouge of Ames (24405), a son of Rouge II's Son, while their dams were sired by Rouge II's Son (18587). From birth until the start of the digestion trial these heifers received the same care and feed. Both were pregnant and in fair condition at the beginning of the experiment, and though No. 298 was rather larger than No. 301, they were a very uniform pair in all other ways.

TABLE VII.—*Description of animals used in trial*

Animal.	Age.			Days bred.	Weight.
	Yr.	*mos.*	*dys.*		*Pounds.*
Heifer 298	1	6	17	63	650
Heifer 301	1	5	27	152	550
Average	1	6	7	108	600

The digestion trial was run for a period of five days preceded by a preliminary period of seven days during which Sudan grass was fed as the only source of nutriment to the heifers. In the preliminary period it was found that 20 pounds per head per day of the hay would be a convenient amount to feed; so this allowance was used throughout the experiment and the material left was weighed back daily.

It has been found that the animals had no special need of being watered twice daily, so the watering was done at the beginning of each 24-hour period and the animals were weighed before and after watering. The attendant collected the feces with a scoop and deposited them in tarred galvanized-iron vessels which were provided with covers.

A composite sample of the hay fed, and one of the orts were made at the end of the trial period. The feces from each heifer were mixed thoroughly and sampled at the end of each 24-hour period, and these samples were air-dried. At the end of the trial an aliquot composite sample was made for the feces produced by each of the heifers during the 5-day trial period.

The composite samples of feces, together with those of hay and orts, were chemically examined according to the official methods.

In Table VIII is given a summary of the hay and water consumed and the feces produced daily by each of the heifers. Only the net consumption of hay is given, and the feces production recorded opposite a daily consumption of hay is the weight of feces produced in the 24-hour period following the day during which the recorded amount of hay was consumed.

TABLE VIII.—*Summary of weight of feed and feces*

Day.	Hay consumed.		Water consumed.		Feces produced.	
	Heifer 298.	Heifer 301.	Heifer 298.	Heifer 301.	Heifer 298.	Heifer 301.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
1	14.2	13.4	24	27	19.1	14.9
2	17.0	16.7	39	29	21.8	20.9
3	13.0	11.6	31	17	24.6	18.4
4	7.9	10.5	28	27	21.4	21.8
5	12.4	14.1	24	26	23.9	19.8
Total	64.5	66.3	146	126	110.8	95.8

The heifers had very similar capacities for hay consumption, the difference in their average daily consumption being only about one-third of a pound (Table IX). Their capacities for water consumption were also very much alike; the heifer which consumed the smaller amount of hay drank on the average 4 pounds more water per day than did the other heifer. The production of feces followed the water consumption very closely, and the heifer which consumed the smaller amount of hay and the greater quantity of water produced the greater weight of feces.

TABLE IX.—*Composition of hay*

Constituent.	Hay offered.	Hay refused.	Hay consumed.
	Per cent.	*Per cent.*	*Per cent.*
Moisture	13.19	11.64	14.01
Dry matter	86.81	88.36	85.99
Protein	5.97	4.10	6.96
Nitrogen-free extract	43.63	42.85	44.04
Crude fiber	28.65	34.50	25.55
Ether extract	1.62	1.08	1.91
Ash	6.94	5.83	7.52

As was to be expected, the hay refused was a little more fibrous than the whole sample. The difference is so small, however, that the digestion coefficients found for the hay consumed will apply equally well to the whole sample.

TABLE X.—*Composition of feces*

Constituent.	Heifer 298.	Heifer 301.
	Per cent.	*Per cent.*
Moisture	82.39	79.13
Dry matter	17.61	20.87
Protein	2.13	2.54
Nitrogen-free extract	8.32	9.74
Crude fiber	4.35	5.24
Ether extract	.47	.54
Ash	2.34	2.81

The analyses given for the feces represent their composition when moist (Table X). Heifer 301, which consumed less hay and more water than did heifer 298, produced the feces with the higher moisture content. The bulk of the feces evidently depends to a large extent on the amount of water consumed.

TABLE XI.—*Summary of weights of nutrients consumed and defecated*

Constituent.	Heifer 298.		Heifer 301.	
	Total nutrients consumed.	Total nutrients defecated.	Total nutrients consumed.	Total nutrients defecated.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*
Dry matter	55.44	19.51	57.03	19.99
Protein	4.51	2.36	4.59	2.43
Nitrogen-free extract	28.42	9.22	29.19	9.33
Crude fiber	16.40	4.82	17.02	5.02
Ether extract	1.24	.52	1.26	.52

Table XI again demonstrates the similarity between the powers of the two heifers for using roughage and also indicates that their powers of digestion are very nearly equal.

TABLE XII.—*Coefficients of digestibility*

Constituent.	Heifer 298.	Heifer 301.	Average.
	Per cent.	*Per cent.*	*Per cent.*
Dry matter	64.8	65.0	64.9
Protein	47.7	47.1	47.4
Nitrogen-free extract	67.6	68.0	67.8
Crude fiber	70.6	70.5	70.6
Ether extract	58.1	58.7	58.4

This shows that the nutrients in Sudan-grass hay are all fairly easily digested. The digestion coefficients range from 47.4 per cent in the case of the protein to 70.6 per cent for the crude fiber, while that for the total dry matter is 64.9 per cent.

A comparison of the work done at this station with that done at the Maryland station shows that the coefficients of digestibility obtained agree fairly closely for most of the nutrients present in Sudan-grass hay (Table XIII).

TABLE XIII.—*Comparison of digestion trials with Sudan-grass hay*

Constituent.	Digestion coefficients (6).		
	Maryland.	Iowa.	Average.
	Per cent.	*Per cent.*	*Per cent.*
Dry matter	60.6	64.9	63.5
Protein	35.4	47.4	43.4
Nitrogen-free extract	63.3	67.8	66.3
Crude fiber	67.1	70.6	69.4
Ether extract	41.2	58.4	52.7

The Iowa results are in all cases higher than those obtained at the Maryland station, but only in the case of the crude protein and ether extract is there a very marked difference. This may, perhaps, be due to differences in the conditions under which the hays were grown, though they are very similar in composition, or more probably to variations in the digestive powers of the animals used. Whatever the factors or factor are that bring about this difference they apparently are selective in their action.

The total and digestible nutrients in 100 pounds of Sudan-grass hay are given in Table XIV.

TABLE XIV.—*Nutrients in 100 pounds of Sudan-grass hay*

Constituent.	Nutrients.	
	Total.	Digestible.
	Pounds.	*Pounds.*
Dry matter	91.6	58.2
Protein	7.7	3.3
Nitrogen-free extract	48.3	32.0
Crude fiber	30.9	21.4
Ether extract	1.8	.9

A comparison of Sudan-grass hay with timothy and millet hay shows that these feeds are very similar in composition. The digestible nutrients in 100 pounds of dry matter of the various feeds (Table XV) have been calculated from Henry and Morrison's tables (*2*), while the digestible true protein and net energy value of 100 pounds of dry matter have been obtained from Armsby's work (*1*).

TABLE XV.—*Digestible nutrients in 100 pounds of dry matter of timothy, millet, and Sudan-grass hay*

Constituent.	Timothy hay.	Millet hay.	Sudan-grass hay.
	Pounds.	*Pounds.*	*Pounds.*
Protein	3.4	5.8	3.6
Carbohydrates	48.4	53.6	58.3
Fat	1.4	2.1	1.0
Total	54.0	64.2	64.2

The data in Table XV show that Sudan-grass hay provides considerably more nutrients than timothy hay, and, though it contains rather less digestible protein than millet hay, it appears to furnish about the same amount of total nutrients. These comparisons are made on the dry matter basis so as to eliminate variations due to changes in the moisture contents of the feeds.

The net energy value of the Sudan-grass hay has been calculated according to Armsby's method (*1*), while the digestible true protein is taken as 75 per cent of the digestible crude protein (Table XVI). These figures show that Sudan-grass hay, though deficient in protein, provides more net energy per 100 pounds of dry matter than hay from timothy or millet.

TABLE XVI.—*Digestible true protein and net energy values per 100 pounds of dry matter in timothy, millet, and Sudan-grass hay*

Item.	Timothy hay.	Millet hay.	Sudan grass hay.
Digestible true protein .... pounds	2.5	4.6	2.7
Net energy value .... therms	48.67	54.80	64.42

SUMMARY

(1) The dry matter of Sudan grass changes slightly in composition from the time of heading until the crop is ripe.

(2) The content of fat and protein increases in the early stages of ripening and decreases later while the changes in the nitrogen-free extract and ash content are in the opposite direction.

(3) Either as a green feed or as hay, Sudan grass is very palatable.

(4) Sudan-grass hay has a comparatively high apparent digestibility.

(5) Sudan-grass hay supplies energy to cattle much more efficiently than it does protein.

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WATER-HOLDING CAPACITIES OF BEDDING MATERIALS FOR LIVE STOCK, AMOUNTS REQUIRED TO BED ANIMALS, AND AMOUNTS OF MANURE SAVED BY THEIR USE

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For 25 years investigators and teachers have been expressing erroneous conclusions as to the relative values of shavings, sawdust, and the straws for bedding live stock. These conclusions are based upon a table showing the relative liquid-absorbing capacities of various substances which appeared in 1893 (*5*).[1] This table, which follows, was adapted from Deherain (*4*).

Absorption of liquids by litter

Kind of litter.	Water retained by 100 kgm. of material after 24 hours.	Quantity of material necessary to replace 100 kgm. of wheat straw.
	Kgm.	*Kgm.*
Wheat straw	220	100
Barley straw	285	77
Oat straw	228	96
Partially decomposed oak leaves	162	136
Peat	500–700	40
Sawdust of poplar wood	435	50
Spent tan bark	400–500	48
Air-dried vegetable mold	50	440

According to this table sawdust has almost twice the water-holding capacity of wheat or oat straw. Not only has this point been repeatedly referred to, but the conclusions have also been drawn that sawdust or shavings will go nearly twice as far as straw as bedding for live stock and will save a much larger portion of the liquid manure.

The above table, or portions of it, have been copied in a number of publications (*2*, *3*, *7*).

On the basis of these figures, the statement is made in Vermont Bulletin 206 that—

> Nine pounds of straw or six pounds of shavings are needed to absorb a cow's 24 hour voidings.

Special Circular 11 of the Dominion of Canada Experimental Farms (*1*) states concerning "dry sawdust and fine shavings" that—

> Their absorptive capacity according to fineness and dryness is from two to four times that of ordinary straw.

[1] Reference is made by number (italic) to "Literature cited," p. 190.

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The values given in this old French table are reversed by tests on the absorptive capacity of oat straw, wheat straw, and shavings, conducted by the writer in the spring of 1917. These tests show that oat straw absorbs 15 to 20 per cent more water than wheat straw and more than twice as much as ordinary commercial mixed shavings. The tests are well substantiated by records kept of the amount of bedding material of the different kinds actually used for different classes of animals.

In order to determine the water-holding capacity of the various materials, weighed quantities (5 to 7 pounds per sack) were sacked loosely and soaked for 12 hours. The sacks were then hung in a room in a barn, and after 5 hours, when dripping had practically ceased, were weighed. They were weighed again after hanging for 24 hours. This test was repeated several times. There was a small variation from time to time, probably due to differences in the particular samples of material obtained and to differences in the rate of evaporation on different days. However, they were relatively the same in each test. In addition to oat straw, wheat straw, and two kinds of shavings which were being used for bedding purposes, some cut oat straw, some mixed sawdust, and some very light, fine white-pine shavings were obtained for these tests. Approximate averages of the results of the tests are given in Table I.

TABLE I.—*Water-holding capacity of litter*

Material.	Water retained by 100 pounds of material after 24 hours.	Relative water-holding power after 24 hours.
	Pounds.	
Oat straw (whole)	250	100.0
Cut oat straw (about ½-inch lengths)	244	97.6
Wheat straw	210	84.0
Mixed shavings from Chicago car load	119	47.6
Mixed shavings from local planing mill	130	52.0
Mixed sawdust from local planing mill	160	64.0
Fine, dry white-pine shavings	185	74.0

It will be noted that whole oat straw retained slightly more water than cut oat straw, about 19 per cent more than wheat straw, and twice as much as the ordinary mixed shavings used for bedding material. Whole oat straw came out slightly above the cut oat straw in every test made.

The fine white-pine shavings and the sawdust retained considerably more water than the coarser mixed shavings, the white-pine shavings retaining three-fourths as much water as oat straw, and the sawdust two-thirds as much. It was impossible to get any accurate comparison between shavings and sawdust of the same kind, because the only kind of sawdust obtainable was mixed. The water-holding capacity of the sawdust varied more than that of any of the other materials.

At the same time that these tests were being made, records were being kept on the relative amounts of oat straw, wheat straw, and shavings required to keep beef cows, dairy cows, and horses bedded, and on the amounts of manure saved by the use of each kind of bedding.

Twelve head of beef cows kept in single stalls were divided into three comparable lots. One lot was bedded with oat straw, one with wheat straw, and one with shavings from a car load bought in Chicago. The wheat-straw and shavings lots were reversed at the middle of the 60-day period. With the dairy cows only two lots were used, 9 head in one lot and 10 head in the other. One lot was bedded with oat straw and the other with shavings from the local planing mill. The lots were reversed at the middle of the 30-day period. Only 3 horses were used: draft mares in box stalls, one bedded with each kind of material. The shavings used were from Chicago.

The animals were all handled in the usual way. The beef cows were out of the barn about 9 hours a day, the dairy cows about 8½ hours, and the horses about 9. No special attempt was made to regulate the amount of bedding used, the men in charge of each barn bedding as usual. The barns were cleaned out daily—that is, the manure and soiled part of the litter were removed. Table II shows the amount of bedding used.

TABLE II.—*Material used in keeping animals bedded*

Animals, period, and material.	Total bedding used.	Amount per animal per day.	Relative amount used.
Horses (1 per lot, 49 days):	*Pounds.*	*Pounds.*	
Oat straw	716	14.61	100
Wheat straw	844	17.22	118
Shavings	1,192	24.32	166
Beef cows (4 per lot, 60 days):			
Oat straw	1,766	7.36	100
Wheat straw	1,928	8.03	109
Shavings	3,207	13.36	182
Dairy cows (9½ per lot, 30 days):			
Oat straw	2,064	7.24	100
Shavings	2,892	10.15	140

In keeping the animals bedded, 40 to 82 per cent more shavings than oat straw and 9 to 18 per cent more wheat straw than oat straw were used. About 15 pounds of oat straw per day was required to keep one of the horses bedded, about 7⅓ pounds to keep one of the cows bedded. The horses were on an earth floor, the cattle on concrete floors. The Ohio Station found that about 7 pounds of straw were needed for steers on concrete floors (*6*).

From the fact that the oat straw was capable of absorbing more liquid than wheat straw or shavings, one might suppose that more of the manure from the animals could be saved by the use of oat straw. In this experiment, however, about the same amount of animal excreta was saved, regardless of the kind of bedding used. To be sure, less oat

straw was used as bedding to save the same amount of excreta. Table III shows the material removed.

TABLE III.—*Amount of manure saved by use of the various litters*

Animals and material.	Total manure removed.	Bedding used.	Excreta removed.	Excreta removed per animal per day.
Horses:	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
Oat straw	3,551	716	2,835	57.8
Wheat straw	3,409	844	2,565	52.3
Shavings	3,925	1,192	2,733	55.7
Beef cows:				
Oat straw	10,227	1,766	8,461	35.2
Wheat straw	10,820	1,928	8,892	37.0
Shavings	12,190	3,207	8,983	37.4
Dairy cows:				
Oat straw	17,831	2,064	15,767	55.3
Shavings	18,214	2,892	15,322	53.7

While there is a variation of several per cent in the amount of excreta saved with each class of animals with the different kinds of bedding, still the variations are not large and are not consistently in favor of any one kind of material. It is evident that there is no very important difference in the amount of excreta saved as a result of the use of one or another of these materials.

SUMMARY

(1) The common belief that the shavings commonly used for bedding live stock have much greater water-holding capacity than straw is erroneous. Oat straw retained approximately twice as much water as shavings and 15 to 20 per cent more than wheat straw.

(2) To keep animals bedded, 40 to 82 per cent more shavings than oat straw and 9 to 18 per cent more wheat straw than oat straw were required.

(3) The amount of animal excreta removed from the barn in the manure was about the same regardless of the kind of bedding material used.

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TWO IMPORTANT INTRODUCED PARASITES OF THE BROWN-TAIL MOTH[1]

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INTRODUCTION

To make clearer the following discussions of two parasites of the brown-tail moth (*Euproctis chrysorrhoea* Linnaeus), a brief summary of the introduction and life cycle of the host species is given here. The brown-tail moth was introduced accidentally from Europe about 1890, probably on nursery stock, and made its first appearance in the vicinity of Boston, Mass. It increased rapidly and soon became widely distributed, extending its ravages over very nearly all New England, and also became established in portions of New Brunswick and Nova Scotia. The destructiveness of the species has diminished very markedly during the past few years, however, due in large part to natural control agents.

The brown-tail moth deposits her eggs during the latter part of July, in an elongate mass of several hundred on the underside of a leaf, commonly of apple (*Malus sylvestris*), pear (*Pyrus communis*), oak (*Quercus* spp.), or wild cherry (*Prunus* spp.), and covers them with a dense layer of brown hair taken from the tuft at the posterior end of her body. These eggs hatch after from two to three weeks and the very small pale brown caterpillars begin feeding in a colony on the terminal leaves, which they tie together with a large amount of fine silk to form a firm web 3 or 4 inches in length. For a considerable period in the early autum the caterpillars feed slightly, coming out of their webs for this purpose from time to time; their growth is very slow, however, and although they molt once or twice they do not attain a length of more than 4 or 5 mm. before becoming dormant for the winter. In the early spring, with the opening of the buds, the caterpillars leave their webs and begin feeding,

[1] The investigations which form the basis for this article were conducted at the Gipsy Moth Laboratory, Melrose Highlands, Mass., under the general direction of Mr. A. F. Burgess. The writer wishes to acknowledge the assistance received from Mr. S. S. Crossman, of the Laboratory, and from many others of the staff who have helped in various phases of the work. The photographs were taken by Mr. H. A. Preston. Determinations of chalcidoid hyperparasites were made by Mr. D. W. Jones, of this Station, and Dr. Robert Matheson, of Cornell University.

still in colonies, near the winter web. But as they become larger they crawl to various parts of the tree or even to different trees nearby, feeding ravenously; they attain their growth about the middle of June, and are then 1½ inches in length. Pupation takes place within loosely spun cocoons fastened in leaves that have been drawn together from the sides, in crevices of the bark, in stone walls, and in other protected places. The moths, issuing after about three weeks, mate, and the females begin depositing their eggs. There is only one generation a year.

IMPORTATION OF THE PARASITES

In the winter of 1905–6, following the first visit by Dr. L. O. Howard, Chief of the Bureau of Entomology, to Europe for the purpose of arranging for the sending to New England of parasites of the gipsy and brown-tail moths, large numbers of the winter webs of the latter species were received at the parasite laboratory, then located at North Saugus, Mass. These webs were placed in special cages, one of which is shown in Plate 19, A. The cage consisted of a large wooden box, capable of holding about 1,000 webs of the brown-tail-moth caterpillars, and having a number of glass tubes projecting from the upper half of one side. Any parasites issuing from the webs would be attracted into the tubes by the light.

Very early in the spring many individuals of *Pteromalus egregius* Foerster, a chalcidoid external parasite of the wintering brown-tail moth larvæ, and also many specimens of *Monodontomerus aereus* Walker, another chalcidoid, that frequently hibernates as an adult in the brown-tail web, entered the tubes. Shortly the brown-tail-moth caterpillars in the cages became active and made their way into the glass tubes; as these became filled, the caterpillars were removed and destroyed, since the probability of their harboring internal parasites seemed slight. However, Mr. E. S. G. Titus, at that time in charge of the work, fed a few of the caterpillars for a time, as an experiment, and secured from them representatives of two parasitic species, both braconids, one of the genus Apanteles and the other belonging to the genus Meteorus. It is these two species with which this paper deals.

REARING AND COLONIZATION OF THE TWO PARASITES

As a result of the discovery by Mr. Titus, the caterpillars from the webs received during succeeding winters were not destroyed upon issuing in the spring, as had been done previously, but were fed for several weeks, so that any internal parasites might be reared. For rearing methods and types of cages used, see Howard and Fiske (*1*).[1] This was continued until 1911, when importations ceased, with the result that some 40,000 cocoons of *Apanteles lacteicolor* and about 1,600 of *Meteorus versicolor*

[1] Reference is made by number (italic) to "Literature cited," p. 205.

were obtained for liberation in brown-tail-moth infestations. That these species are widely distributed in Europe was shown by the recovery of both parasites from webs that had been sent from the following countries: France, Netherlands, Germany, Russia, especially southern Russia, Austria, Switzerland, and Italy. Once introduced into New England, both species became quickly established, so that after 1911 a large amount of material for colonization was obtained from caterpillars collected in local infestations. So rapid has been the spread of the parasites that, although up to the present only 150 colonies of the Apanteles and 20 of the Meteorus have been placed in 135 and 18 towns, respectively, no further colonization is necessary. Both parasites have been recovered from practically the entire brown-tail moth area, either by rearing from the brown-tail moth larvæ in the early spring or by dissection from hibernating caterpillars.

In making collections of brown-tail-moth webs for the recovery of these parasites and to obtain material for colonization, whenever possible 105 webs have been taken in each of a considerable number of towns some time during the winter and placed in cold storage until spring. One hundred of these were then placed in one of the large rearing trays (Pl. 19, B), and the caterpillars fed. The foliage was placed upon mosquito netting that had been laid over the webs. The purpose of this was to draw the caterpillars away from their webs, so that the latter could be removed readily and destroyed by rolling back the netting with foliage and larvæ. This greatly facilitated the picking over of the webs for the cocoons of the parasites. The remaining five webs were placed in small individual trays (Pl. 19, C). The caterpillars in each of these single-web trays were counted, and since a count of the Apanteles and Meteorus cocoons removed later was also made, the extent of parasitism by these two species at each locality was determined.

When dissections were made for the recovery of the parasites, as in 1917 and 1918, the work was done early in the year while the caterpillars were still dormant. In 1917, the first year of systematic dissection work for the recovery of brown-tail-moth parasites, 5 webs were taken from each of the collections that had been made the preceding fall or during the winter; from each of these webs 50 caterpillars were taken at random and dissected under the binocular microscope. Later it seemed that dissecting a smaller number of caterpillars from a larger number of webs would give a better representation of actual parasite conditions. Accordingly, this year (1918) 20 brown-tail-moth larvæ were dissected from each of 10 webs. This method of determining the extent of parasitism by making dissections probably gives more accurate data than rearing, since the somewhat unnatural conditions under which the caterpillars are fed in the spring must prevent the issuance of some of the parasites. Moreover, the dissections give data on the extent of

competition between the different parasitic species hibernating in the brown-tail-moth larvæ. Still other information obtained by opening the webs and dissecting caterpillars is that on "winter-killing" among the brown-tail-moth larvæ, and the relation of parasitism to this phenomenon. Very low temperatures act as an important control agent in the more northern parts of the brown-tail-moth area in Maine, New Hampshire, Vermont, and parts of Massachusetts. It might be expected that caterpillars infested by parasites would succumb more quickly to the cold, and that thus the low temperatures would act as a proportionately greater check upon the parasites than upon the brown-tail moth larvæ. Fortunately this does not appear to be the case. The parasitism in webs containing a large percentage of dead caterpillars is scarcely, if at all, less than in webs from the same locality with few dead larvæ. Dead caterpillars, as found in the webs, are usually dried up and unfit for dissection, but when dissections have been possible the dead larvæ showed no abnormally great parasitism. The probable reason for this is that the hibernating parasites are very small and have made no great inroads upon the reserve food of the host larvæ, their feeding having been slight at most and extended over a considerable period, so that the caterpillars have not been materially weakened.

APANTELES LACTEICOLOR VIERECK

The species of Apanteles that hibernates in the young caterpillars of the brown-tail moth was described by Viereck in 1911 (7, *p.* 475) from material reared at the Gipsy-Moth Laboratory, Melrose Highlands, Mass., as "*Apanteles lacteicolor*." That so widespread and so general a parasite of the brown-tail-moth caterpillars in Europe should not have been described before is somewhat surprising, and it may yet be found that the insect has been described, but too imperfectly to be recognized thereby. Meanwhile the name "*Apanteles lacteicolor* Viereck" must stand.

As Viereck's description is very brief, a fuller characterization is given herewith.

Male and female.—Length 2.5 mm. Black; head including the antennæ black, covered with a short sericeous pubescence; vertex, front, face, and clypeus all finely punctate; eyes hairy; female antennæ as long as the body, those of the male longer.

Thorax: Mesoscutum densely, rather deeply punctate; scutel more shining, with sparse very shallow punctures, slightly convex; tegulæ black. The propodeum is very distinct from that of other species of the genus in that it has on the apical two-thirds three, one median and two lateral, large shining areas, and a less distinctly margined and less smooth almost circular area on each side near the base; the median area is pentagonal, while the apical lateral areas are subtrapezoidal, and extend slightly beyond the apex of the median area, reaching the posterior margin of the propodeum. The wings have the veins generally pale, with the costa and stigma brown in the female and only the outline of the stigma brown in the male; the exterior vein of the first cubital cell is only slightly angled a little below the middle. Legs:

The anterior pair have the coxæ black, the trochanters and extreme base of the femora dusky, the remainder of the femora, the tibiæ, and the tarsi yellowish; the middle legs have the coxæ, the trochanters, and the basal two-thirds of the femora black, and the apical half of the tibiæ dusky, the base of the tibiæ and the tarsi yellow; the posterior pair have the coxæ, trochanters, femora, and the apical half of the tibiæ black, and the tarsi blackish, except the extreme base of the basal segment, which is yellowish.

Abdomen: Somewhat shorter than thorax, entirely black; first and second tergites coarsely rugose; the first long, scarcely wider at apex than at base, and usually with a small, indistinct, shining median fovea on its apical half; the second tergite is transverse, three to four times as broad as long down the middle, the posterior margin arcuate; the broad lateral membranous margins on the apical one-third of the first tergite, and along the second blackish; third tergite and beyond smooth and shining. The ovipositor is long, almost half as long as the abdomen.

SEASONAL AND LIFE HISTORY OF APANTELES LACTEICOLOR

THE EGG

The female of *Apanteles lacteicolor* (Pl. 20, A) oviposits in first and second stage brown-tail-moth caterpillars during the month of August. The very small larvæ, those being but two or three days from the egg, are preferred, often being attacked while still on the egg cluster, before they have fed at all. The egg of the parasite (Pl. 20, B) is a minute transparent object, measuring but 0.35 mm. in length, including the stalk at the end opposite the micropile. No particular part of the body of the host is selected for oviposition, the attack being merely a nervous thrust, requiring about one second, into any part of the caterpillar. Only a single egg is deposited at one oviposition, and usually only one egg is placed in a caterpillar. The parasite will oviposit from a dozen to 25 times in quick succession if hosts are available, and will then rest for a period, sometimes for a day or two, before depositing more eggs. Oviposition may extend over a period of several weeks, and a single female may attack upwards of 300 caterpillars, although under field conditions the average appears to be much lower, probably due in large part to the fact that the insect is delicate and rather short lived. In the laboratory one female, over a period of two weeks, oviposited in 320 larvæ, placing two and even three eggs in some of these. That an egg was being deposited with each thrust of the ovipositor was determined by dissecting from time to time caterpillars that had been attacked by the parasite.

HIBERNATING LARVA OF APANTELES LACTEICOLOR

The egg of *Apanteles lacteicolor*, having increased somewhat in size, hatches after about three days, and the young parasitic larva, free in the body cavity of its host, feeds slightly on the fat and lymph there, merely keeping pace with the very slow development of the caterpillar prior to hibernation. The position of this first-stage larva within its host varies, but more commonly the young *A. lacteicolor* is found in the posterior half of the body. As dissected from the hibernating

brown-tail-moth caterpillar, the Apanteles larva is minute and transparent, slightly less than 0.5 mm. in length, and possessing at its caudal end a curious bladder-like organ, commonly referred to as the anal vesicle, and beneath this a prominent fleshy horn projecting downward. On the dorsum of each of the last nine segments of the body is a transverse row of very indistinct, short, though rather stout, backward-projecting spines. When first removed from the caterpillar, the position usually taken by the parasitic larva is that shown in Plate 20, C, the body being curved so that the caudal horn touches or passes across the head. In this stage no tracheal system is visible.

ANAL VESICLE OF APANTELES LACTEICOLOR

The parasitic larvæ of the subfamily to which this species belongs, the Microgasterinae, have at the caudal end of the body a bladder-like structure, called the "anal vesicle," which has been the subject of much discussion, particularly in Europe, by such entomologists as Kulagin, Seurat, and Weissenberg. Weissenberg (*8*, *9*), working principally on *Apanteles glomeratus* Linnaeus, formed a number of conclusions on the structure and function of the organ and summarized very well all the work that had been done upon this subject, besides giving the results of his own investigations. It has been determined definitely that the anal vesicle of these larvæ consists merely of a portion of the hind gut which has been evaginated; all but the extreme posterior part of this section of the intestinal tract is concerned, being turned inside out so that the blind end of the midintestine is on the outer ventral surface of the vesicle. The vesicle is present in all endoparasitic stages of *Apanteles* spp., but is reinvaginated very shortly before the larva issues to spin its cocoon. Its function is not so definitely known, but there seem to be two important uses. The fact that the tracheal system of the parasite is not developed until the last endoparasitic stage, when the vesicle begins to be retracted, strongly suggests respiration as an important function, and the delicate structure of the organ would emphasize this. Frequently, when dissecting brown-tail-moth caterpillars during the winter, the writer has found hibernating first-stage larvæ of *A. lacteicolor* in which the hind intestine was not at all evaginated but could be distinctly seen within the larva (Pl. 20, C). On several occasions while such a larva, placed in a drop of water, was under observation, the hind intestine was seen to be slowly pushed out through the anal opening to form the vesicle. While inactive within the host and under a comparatively low temperature, the respiration of the parasite is reduced to the minimum, and the slight respiration that does take place may go on through the body wall. When removed from the caterpillar and placed in water, the parasite becomes active and respiration increases. That at this time the vesicle should be formed supports the theory that an important function of the anal vesicle is respiration. However, Weissenberg (*9*) thinks, as a result of

a series of homologues with the intestinal tracts of other parasitic larvæ, that a yet more important function is excretion. For the present the matter must rest here, but further study may throw more light upon the function of this curious structure.

COMPETITION WITH OTHER PARASITES WINTERING IN THE BROWN-TAIL-MOTH CATERPILLARS

Since there are two other parasitic species that pass the winter within the small brown-tail-moth caterpillars there is naturally some competition between the three. These other species are *Zygobothria nidicola* Townsend, a fly of the family Tachinidae, and *Meteorus versicolor* Wesmael, the other parasite discussed in this paper. It was determined from over 13,000 dissections of hibernating brown-tail-moth caterpillars that whenever *A. lacteicolor* enters into competition with either or both of these species it wins out; the other parasite or parasites present are killed before midwinter, evidently as the result of some toxic action induced by the Apanteles larva. Even when two larvæ of *A. lacteicolor* occur in the same host, only one of these normally completes its development. Some very interesting cases of competition were encountered in the course of the dissecting: In one caterpillar were found two larvæ of *A. lacteicolor*, one of them dead, five dead Zygobothria maggots more or less encysted and no longer occupying their normal position in the fore intestine of the caterpillar, and three eggs of *Meteorus versicolor*, the development of which had been arrested; another caterpillar contained a living larva of *A. lacteicolor*, nine dead Zygobothria maggots, and one partly developed egg of *M. versicolor*, the embryo dead. Many similar cases were encountered, but in no case was a dead Apanteles larva found in the same caterpillar with a living larva of some other parasitic species. This ability to win out over the species of parasites hibernating within the brown-tail-moth caterpillar strengthens considerably the position of *A. lacteicolor*.

LATER ENDOPARASITIC LIFE

In May, when the brown-tail-moth caterpillars resume feeding, the small larvæ of *A. lacteicolor* within these caterpillars likewise become active and begin in earnest the task of destroying their hosts. The parasitic larva develops very rapidly; after a day or two of feeding it attains the length of 1 millimeter, or slightly more (Pl. 20, D), and passes into the second stage, which differs from the first principally in that the mandibles, or structures corresponding to the mandibles, are bidentate and not chitinized, whereas in the first stage they were simple and heavily chitinized (Pl. 20, E). The anal vesicle is much more in evidence, being proportionately larger, but the horn beneath, so prominent in the first stage, is scarcely noticeable, not having increased in size at all. The almost invariable position of *A. lacteicolor* now is in the posterior half of the body of its host, the parasite being longitudinally disposed, its

head directed toward the caudal end of the caterpillar. After 2 or 3 days the parasite passes into the third stage (Pl. 21, A); the mandibles of this stage differ markedly from those of either of the other stages, being much longer and pectinate (Pl. 20, E). In the third stage the larva possesses a tracheal system, not evident before. In this stage, too, the anal vesicle begins to be reinvaginated, being gradually drawn back into the body of the larva, so that, when the parasite issues, no vesicle can be seen.

The infested brown-tail-moth caterpillars are very noticeably retarded and do not get beyond the stage in which they hibernated. Death occurs in from 7 to 12 days after they have begun feeding, and very shortly the full-grown parasitic larva issues.

The species of Apanteles commonly do not kill their hosts upon issuing, the latter sometimes remaining alive two weeks or more. The death of the victims of *A. lacteicolor* then, just prior to issuance of the parasite, is interesting. Just how this death of the host is brought about is not certainly known; but the writer found, on dissecting caterpillars from which this parasite had just issued, that the central nervous system in the posterior part of the body was entirely destroyed, while in various caterpillars, still living, deserted by other species of Apanteles, no such injury had taken place. That in the former case destruction of the nervous system occurs not more than a few hours before the issuance of the parasite, was determined as the result of dissecting a number of brown-tail-moth caterpillars containing *A. lacteicolor* larvæ almost ready to issue, these caterpillars being still alive; the nervous system in these cases had not yet been injured. It seems very probable, after these observations, that the destruction of the nervous system by the larvæ of *A. lacteicolor* is responsible for the early death of the hosts of this parasite.

COCOON OF APANTELES LACTEICOLOR

Directly upon issuing from its host, the larva of *A. lacteicolor* begins spinning its cocoon, completing this after three hours or more. The process of spinning consists of a continuous looping of the silken thread as this is spun out, and a careful fastening of these loops; the larva finds it necessary, in the course of this work, to reverse its position many times. When complete the cocoon is pure white, oblong-cylindrical in form, 4 to 4.5 mm. in length, and surrounded by a small amount of loose silk. The cocoons of the wintering generation are commonly found in the webs of the brown-tail moth caterpillars, while those of the summer generations occur on the underside of leaves, in crevices of the bark, etc.

Changes within the cocoon are rapid. From 18 to 24 hours after spinning has ceased, the waste matter that has accumulated in the mid-intestine during endoparasitic life (the caudal end of the midintestine is closed during this period) is excreted and is forced to the end of the cocoon. Pupation takes place about 48 hours after the larva has ceased

spinning, and the old larval skin is pushed back upon the excrement previously voided. Gradually the pupa (Pl. 21, B), yellowish white at first, blackens; then the pupal skin is cast, and the adult parasite emerges after first cutting out a perfectly circular lid at one end of the cocoon. The total length of the period spent within the cocoon is from 5 to 8 days.

The adults of the first generation of *A. lacteicolor* are found issuing from about the 20th of May to the middle of June in New England. Mating will take place almost at once, within 24 hours after emergence, and oviposition may begin within 48 hours. Laboratory experiments have shown females of this species unwilling to oviposit during the first 24 hours, but they will do so very readily shortly after this. As is true with many parasites, fertilization is not necessary for reproduction, but unfertilized females produce only males.

SUMMER HOSTS OF APANTELES LACTEICOLOR

Considerable effort has been expended by the writer to determine in what hosts this parasite passes the summer. The species has been reared frequently from small gipsy-moth caterpillars at the laboratory, and Howard (*2*, *3*) emphasized the importance of the parasitism upon this species. The writer's observations in the field and experiments at the laboratory have convinced him that wherever brown-tail-moth caterpillars occur in sufficient numbers to insure the presence of a fair proportion of *A. lacteicolor*, the parasitism upon the small gipsy-moth caterpillars is considerable. These are attacked in the first or second stage and are killed by the parasite before they have passed the third (Pl. 21, C). They are greatly retarded in their development when infested by the Apanteles, and when the development of the parasite is nearly complete, the caterpillars seek places of concealment on the lower side of leaves and limbs, in crevices of the bark, etc. Hence they are easily overlooked by men collecting caterpillars to determine the extent of parasitism. So far as the writer has been able to determine, the gipsy moth is the only host, acceptable to *A. lacteicolor*, which is available at the time of the appearance of the adult parasites of the first generation. Where the gipsy moth does not occur the Apanteles females evidently do not oviposit for several weeks, until various native host species, which this parasite will attack, appear. One much retarded caterpillar of *Malacosoma americana* Fabricius was attacked by *A. lacteicolor*, but no reproduction was secured, although, as was found later by dissection, an egg had been deposited. This species is normally too far advanced by the time of the appearance of *A. lacteicolor* to serve as a host of this parasite.

The total period required for the development from egg to adult, in the case of the summer generations, averages 19 to 20 days, and it is during the last weeks of June and in early July that adults of the brood on the gipsy-moth caterpillars emerge.

Between this date and the time of oviposition in the hibernating caterpillars of the brown-tail moth there is a period of more than a month, or ample time for another generation. Furthermore, at this time there are in the field a number of species that would seem to be desirable hosts, including the caterpillars of *Hemerocampa leucostigma* Smith and Abbot, *Notolophus antiqua* Linnaeus, *Datana ministra* Drury, *Hyphantria cunea* Drury, *Apatela hasta* Guenée, and others. Experiments have been carried on in the laboratory with a number of known and unknown species, the caterpillars being reared from eggs and hence parasite-free at the time of their subjection to *A. lacteicolor.* In addition, field collections of first and second stage larvæ of various possible host species have been made, and these reared for the recovery of the Apanteles. In the laboratory reproduction has been secured upon *Apatela hasta* Guenée, *Schizura unicornis* Smith and Abbot, *Hemerocampa leucostigma* Smith and Abbot, and an undetermined arctiid. First-stage larvæ of *Apatela hasta* were very eagerly attacked by *Apanteles lacteicolor,* as were also first-stage larvæ of the *Schizura unicornis* and of the undetermined arctiid. In 1910 *A. lacteicolor* was recovered in the field from *Datana ministra* and *Hyphantria cunea* (*1, p. 289*), and during the past summer the writer has recovered it from *Apatela hasta*, a noctuid not uncommon upon wild black cherry and the species upon which *A. lacteicolor* reproduced so readily in the laboratory. Further evidence of the probable importance of *Apatela hasta* as a host of *A. lacteicolor* was the collection of cocoons of the parasite, during the last week of July, upon wild black cherry where only *A. hasta* was present in numbers. This species can be found in virtually all stages throughout the month of July, and, to judge from observations in the field and laboratory, the writer believes it to be an admirable host for tiding *A. lacteicolor* over the period elapsing before the brown-tail-moth caterpillars that are to carry the parasite over the winter become available.

ECONOMIC IMPORTANCE OF APANTELES LACTEICOLOR

As a control agent *A. lacteicolor* must take high rank. First, it is a very effective parasite of the brown-tail moth, as high as 20 to 25 per cent of the larvæ of a web often being parasitized by this species. Then the facts that there are several generations annually, and that it is a parasite of more or less importance upon the gipsy moth and upon certain native injurious species, add to its value. In addition, *A. lacteicolor* destroys its hosts in the early stages, and thus prevents any considerable feeding by the individuals parasitized, since these are very greatly retarded. Plate 21, D, shows a parasitized and an unparasitized caterpillar of an undetermined arctiid hatched on the same day from the same egg mass and similarly fed. Actual measurements in a number of cases showed that the individuals parasitized by *A. lacteicolor* eat, on the average, about one-fourth as much foliage as caterpillars of the same species and the same

age not parasitized. These factors combine to make *A. lacteicolor* a parasite of considerable importance.

The weak point in the life cycle of the parasite is its evident dependence upon the brown-tail moth for hibernation. This species is now on the decadence, and with it *A. lacteicolor* is becoming less abundant, thus reducing very materially the parasitism upon the gipsy moth and native hosts.

SECONDARY PARASITISM UPON APANTELES LACTEICOLOR

Since the cocoons of the first generation of *A. lacteicolor* occur for the most part within the webs of the brown-tail moth, they are protected from secondary parasitism to a great extent, and a very small percentage of these cocoons is parasitized. Those of the later generations, however, are more accessible to secondaries, and among these parasitism runs quite high. The hyperparasitic species reared from *A. lacteicolor* include the following: *Monodontomerus aereus* Walker, *Pteromalus egregius* Foerster, *Dibrachys boucheanus* Ratzeburg, *Dimmockia* sp., *Habrocytus* sp., *Pezomachus* sp., and two species of Hemiteles.

METEORUS VERSICOLOR WESMAEL.

The species of Meteorus wintering in the hibernating brown-tail-moth caterpillars was described by Wesmael in 1835 as "*Meteorus versicolor.*" It is an extremely variable form, and a number of varieties, which may or may not be good, have been founded on color differences. Following is a redescription of the species based on the examination of many specimens bred from European as well as from local material.

Length 3.5–5 mm. General color honey-yellow; however, there is great variation in color: Some specimens are entirely yellowish, with no black markings whatever; while others have the propodeum and most of the dorsum of the abdomen black; all gradations between these forms can be found.

Head transverse, yellow; antennæ yellowish to brownish; eyes bluish to black; stemmaticum sometimes black; mandibles yellowish, except the extreme tips, which are brownish; palpi yellowish.

Thorax: Mostly honey-yellow; prothorax, mesothorax, and scutellum yellow, except occasionally the lobes of mesothorax dusky; the mesothoracic lobes feebly punctate, the parapsidal grooves broad, well marked, and ending posteriorly in a broad, depressed, roughened area, which extends to the apex of the mesoscutum; suture at base of scutellum deeply foveate. Propodeum variable, but usually at least somewhat discolored, and often entirely blackish; metapleuræ deep honey-yellow, even when propodeum is entirely black; propodeum not sloping from base to apex, the posterior declivity abrupt. Wings: Base of the costa brownish, the rest of the veins and the stigma pale; cubitus beyond the second cubital cell subobsolete; the second cubital cell quadrate; recurrent vein variable, entering the first cubital cell or interstitial with the first transverse cubitus. Submedian cell very distinctly longer than the median. Legs: Entirely honey-yellow, except sometimes slightly dusky on the apex of the hind coxæ, the apex of the hind femora, the apex of the hind tibiæ, and the hind tarsi.

Abdomen: Not or scarcely longer than the thorax; varying from entirely yellowish to largely blackish; segment 1 is longitudinally aciculated on the apical half, and does

not possess the elongate fossæ found in some species of the genus; segment 2 and beyond very smooth and shining; segment 1 nearly as long as the remainder of the abdomen; its basal half always pale yellowish, its apical half usually blackish, except extreme apical margin which is yellowish; the second segment commonly with a large blackish or brownish spot on each side, the two occasionally merging, but usually leaving the middle of the segment yellow; beyond the second segment the abdomen is more or less blackish, sometimes entirely black. Ovipositor about one-half the length of the abdomen.

SEASONAL AND LIFE HISTORY OF METEORUS VERSICOLOR.

THE EGG.

Like *Apanteles lacteicolor*, *M. versicolor* oviposits in the small brown-tail-moth caterpillars during August and early September. Like *A. lacteicolor*, too, *M. versicolor* deposits only a single egg with each thrust of the ovipositor, although a number of caterpillars may be attacked within a very few minutes. Oviposition by this species is very deliberate. The parasite (Pl. 22, A), slowly bending the abdomen downward and forward so that the ovipositor is parallel with the venter and projects between the anterior legs, advances stealthily toward her victim. On reaching the larva she remains perfectly motionless for a moment or two, apparently waiting for the caterpillar to make some movement; then, with a quick forward thrust, she inserts the ovipositor, and almost at once withdraws it again, having left an egg in the body of the caterpillar. The egg (Pl. 22, B) is a minute, pale brownish-yellow, oval body, about 0.2 mm. in length, with a prominent stalk, 0.1 mm. long, at the end opposite the micropile; the surface is marked off in minute hexagonal areas. It increases much in size, until 5 or 6 days after deposition, when the larva is ready to issue (Pl. 22, C), it measures 0.6 to 0.75 mm. in length, exclusive of the stalk, which has not increased in size, and about 0.55 mm. in breadth. Shortly before it is ready to issue from the egg the larva of *M. versicolor* becomes very active, as can be observed easily under magnification, the chorion now being transparent. After lashing about with its long caudal appendage for some little time, it finally breaks out and floats free in the body cavity of its host.

HIBERNATING LARVA OF METEORUS VERSICOLOR

At the time the larva issues from the egg its total length is about 1.5 mm. The striking features of this stage are the long caudal horn, 0.6 mm. in length, and the large, brown, heavily chitinized head capsule containing a pair of strong curved mandibles. The caudal appendage is merely a fleshy extension, obviously to aid the larva in getting out of the egg and later in locomotion. It does not have the significance of the anal vesicle of the larva of *A. lacteicolor*, for in *M. versicolor* there is no need of a special respiratory device, the tracheal system being already developed in the first-stage larva. This larva feeds very slightly in the fall, increasing scarcely at all in size, and passes the winter in the

first stage within the body cavity of its host. So far as the writer has been able to determine, *M. versicolor* hibernates only in the brown-tail-moth caterpillars in New England.

When dissections were being made of hibernating brown-tail-moth larvæ in late winter, partly developed eggs of *M. versicolor* were often found, and the writer at first supposed that the species occasionally might go through the winter in this way. But later it was observed that always, when such an egg is found in a hibernating brown-tail-moth caterpillar, there occurs with it a first-stage larva of *A. lacteicolor*. The latter was evidently there first, and was able to prevent the complete development of the egg of *M. versicolor*, perhaps through the secretion of some toxic substance which killed the embryo. That the embryo is actually dead can usually be determined on close examination, provided that development has gone sufficiently far.

LATER ENDOPARASITIC STAGES OF METEORUS VERSICOLOR

In the spring when the brown-tail-moth caterpillars begin feeding the larvæ of *M. versicolor* within some of them also become active, and after from 10 to 14 days the cocoons of the parasite appear. Development of the larva is not as rapid as in the case of *A. lacteicolor*, for the feeding of the parasite does not prevent the host from molting once in the spring. The first-stage larva of *M. versicolor* attains a total length of about 2 mm. and passes into the second stage about 3 days after resuming activity. The second-stage larva no longer possesses the brown, heavily chitinized head capsule and the strong curved mandibles; the mandibles, or what correspond to the mandibles (Pl. 22, D), are exceedingly difficult to find, not being chitinized. The anal appendage, too, is no longer so much in evidence. The length reached in this stage is about 4 mm., and the duration of the stage is about 4 days. The third stage, extending over a period of from 2 to 3 days, differs from the preceding stage principally in that the mandibles are chitinized, the anal appendage is reduced to a short spur, and the size is slightly larger. When full grown (Pl. 21, E) the parasite measures 5 to 6 mm. in length, is cylindrical, and yellowish. It does not kill its host before emerging, as does *A. lacteicolor*, but leaves the latter to writhe for 24 hours or more.

COCOON OF METEORUS VERSICOLOR

Unlike *A. lacteicolor*, the larva of *M. versicolor* does not begin spinning at once on issuing from its host, but commonly crawls some little distance along a twig or branch and then, suspending itself by a strong thread, which it has spun and made secure, forms its cocoon, which is elongate-oval but somewhat attenuated at both ends, and brown. Within the cocoon the head of the parasite is directed downward; the excrement which accumulated during endoparasitic life is pushed to the upper end about 36 hours after spinning has ceased, and a day or two

later the last larval skin is pushed back upon this. The pupal period requires from 4 to 6 days, bringing the total time spent within the cocoon to from 7 to 9 days. The emergence of the adult is through an opening made by cutting off a circular lid at the lower end; thus the cocoon is left hanging in mid-air, even after the parasite has gone (Pl. 21, G). Scheidter (5) in recording his observations on *M. versicolor* in Europe, states that the period from the issuance from the host to emergence from the cocoon is 13 to 14 days, while the pupal period alone is 9 days; but in no case that has come under the writer's observation has the period spent within the cocoon been as long as this.

SUMMER HOSTS OF METEORUS VERSICOLOR

The adults of the first generation emerge during the first two or three weeks of June. Mating takes place very soon after issuance, and the females begin ovipositing. Here, again, fertilization is not necessary for reproduction, but, as is true with *A. lacteicolor*, unfertilized females produce only males.

In Europe Schmiedeknecht (*6, p. 223*) records *M. versicolor* from the following hosts: *Laria v-nigrum* Müller, *Asteroscopus sphinx* Hufnagel, *Bombyx neustria* Linnaeus, *B. lanestris* Linnaeus, *Triphaena pronuba* Linnaeus, *Geometra papilionaria* Linnaeus, *Eupithecia exiguata* Hübner, and *Argyresthia nitidella* Fabricius. In New England the adult parasites of the first generation evidently prefer the last two stages of the brown-tail-moth caterpillars for oviposition. Only occasionally are gipsy-moth caterpillars attacked by this species; *M. versicolor* oviposits very eagerly in *Hemerocampa leucostigma* and *Notolophus antiqua*, however; *Hyphantria cunea* has also been recorded as a host (*1, p. 289*). This parasite has, besides, been observed frequently to insert its ovipositor into a larva in which dissection or rearing showed that no egg had been deposited. A number of specimens of *Alsophila pometaria* Harris were apparently oviposited in by *M. versicolor*, but no parasite was obtained on rearing, which was somewhat surprising, since the parasite has been recorded in Europe from *Eupithecia exiguata*, which also is one of the geometrid subfamily Hydriomeninae. Caterpillars of *Phigalia titea* Cramer (a geometrid), of *Xylina antennata* Walker (a noctuid), and of several species of Tortricidae, as well as larvæ of a tenthredinid, were apparently oviposited in, but rearing and dissection showed that no eggs had been deposited. When even a membracid nymph was introduced into a vial containing a female of *M. versicolor*, the parasite advanced toward the hemipteron with ovipositor projecting forward between the front legs. Evidently *M. versicolor* often attacks from some motive other than that of oviposition.

There is unquestionably at least a partial third generation on various native hosts, particularly upon the species of Hemerocampa, Notolophus, Hyphantria, and other closely allied forms, early stages of which are in

the field during July. The adults of this generation, together with those of the other generations that have lived over, oviposit in the small brown-tail-moth caterpillars during the early autumn. The adults of *M. versicolor*, particularly the females, are much more rugged than those of *A. lacteicolor*, and often live many weeks, even two or three months, so that occasionally females of the first generation may attack the small brown-tail-moth caterpillars in the fall.

ECONOMIC IMPORTANCE OF METEORUS VERSICOLOR

As a parasite of the hibernating brown-tail-moth caterpillars *M. versicolor* is much inferior to *A. lacteicolor*, destroying, on the whole, only a small percentage of them. On some occasions cocoons of *M. versicolor* have been found in enormous numbers in heavy brown-tail-moth infestations, but these cases are not common. Moreover, the parasitism upon the nearly full-grown brown-tail-moth larvæ is slight, and that upon native caterpillars appears to be almost insignificant. The reasons for the lesser importance of this parasite are probably in large part the dependency of the species upon the brown-tail moth for hibernation and the extremely heavy parasitism by secondaries. Fully 50 to 75 per cent of the cocoons of *M. versicolor* are parasitized by various chalcidoids and ichneumonids, which recalls Riley's note (*4, p. 532*) following his description of *Meteorus hyphantriae*, where he states that—

> . . . of 450 cocoons collected 84 per cent were hyperparasitized.

Among the secondary parasites reared from *M. versicolor* were representatives of the following chalcidoid genera: Eupelmus, Spilochalcis, Dibrachys, Hypopteromalus; and of the ichneumonid genera Pezomachus and Hemiteles. Still another factor contributing to lessen the importance of the Meteorus is the failure of many larvæ to transform to pupæ after they have spun cocoons; the percentage of Meteorus cocoons which give forth neither primaries nor hyperparasites is very high. Furthermore, the larva of *A. lacteicolor*, whenever it occurs in the same hibernating brown-tail-moth caterpillar with *M. versicolor*, causes the death of the latter. All these checks upon its development and increase combine to make *M. versicolor* a parasite of lesser importance.

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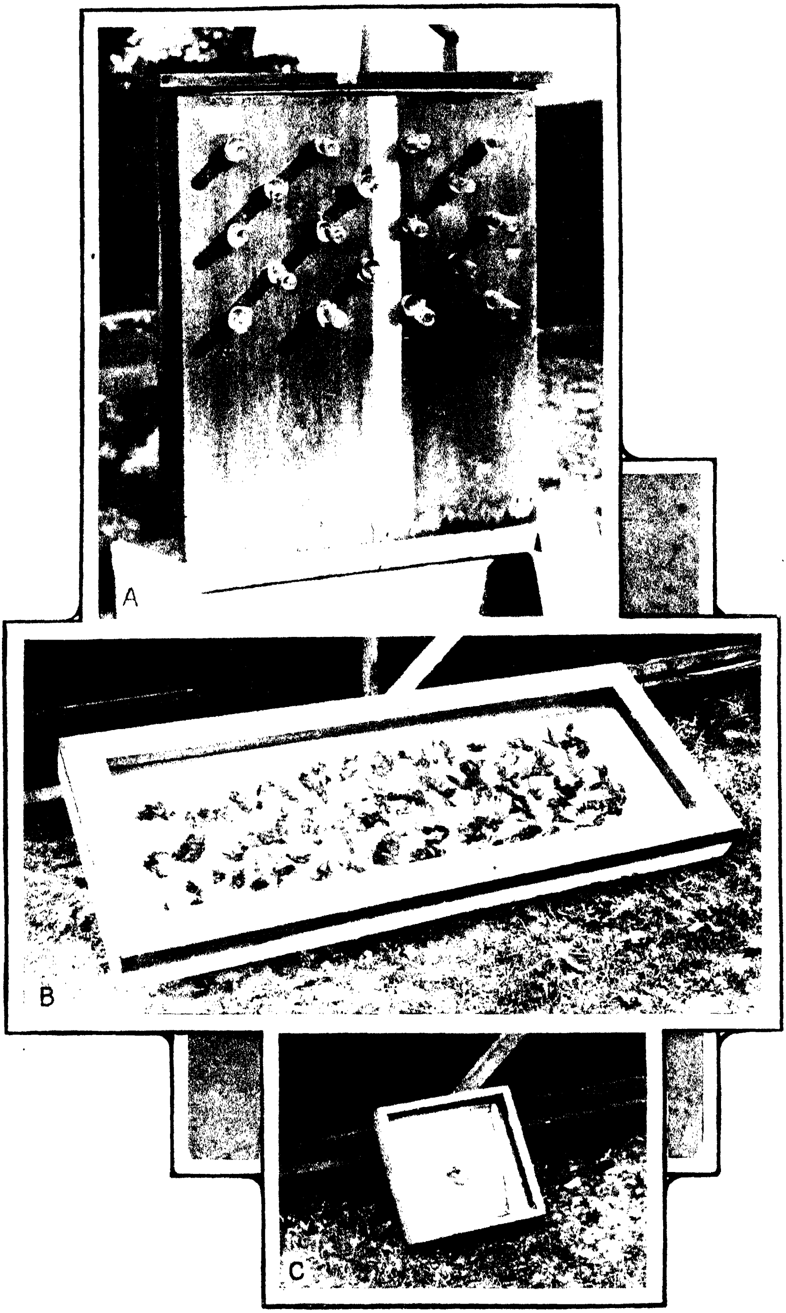
PLATE 19

A.—Large wooden cage used for rearing parasites from imported brown-tail-moth webs. (Designed by Messrs. Howard and Fiske.)

B.—Large rearing tray with cloth bottom, designed by Mr. W. F. Fiske, and used for rearing *Apanteles lacteicolor* and *Meteorus versicolor* from brown-tail-moth caterpillars. Webs are shown on bottom of tray.

C.—Small single-web rearing tray with paraffin-paper bottom.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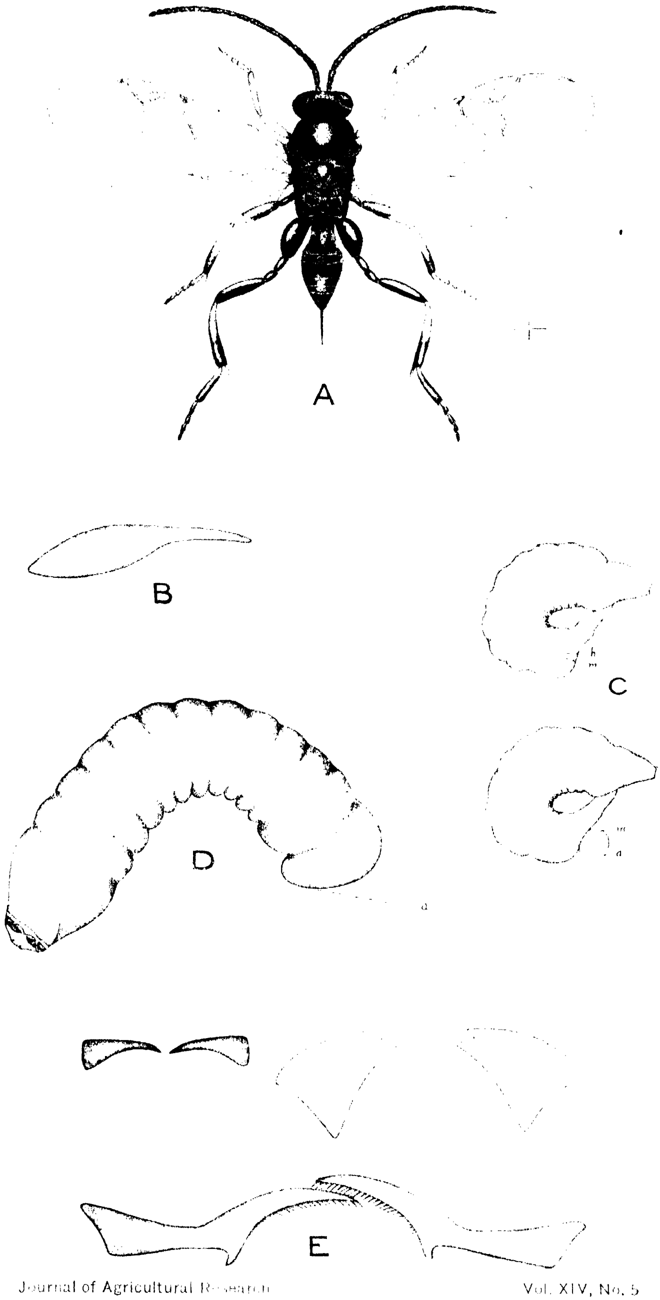
A
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E

PLATE 20

Apanteles lacteicolor:

A.—Adult female. Much enlarged.

B.—Egg. Much enlarged.

C.—Hibernating (first stage) larva, before (above) and after (below) evagination of hind intestine: *h*, Hind intestine; *m*, place of attachment of midintestine to hind intestine; *a*, anal vesicle. Much enlarged.

D.—First-stage larva after feeding in spring, ready to pass into second stage; dorsal view: *a*, Anal vesicle. Much enlarged.

E.—Larval mandibles: Upper left, first stage; upper right, second stage; below, third stage. The mandibles of first and third stages are chitinized; those of the second stage not chitinized. Much enlarged.

PLATE 21

A.—*Apanteles lacteicolor:* Third-stage larva. Anal vesicle still present. ×5.

B.—*A. lacteicolor:* Pupa. ×5.

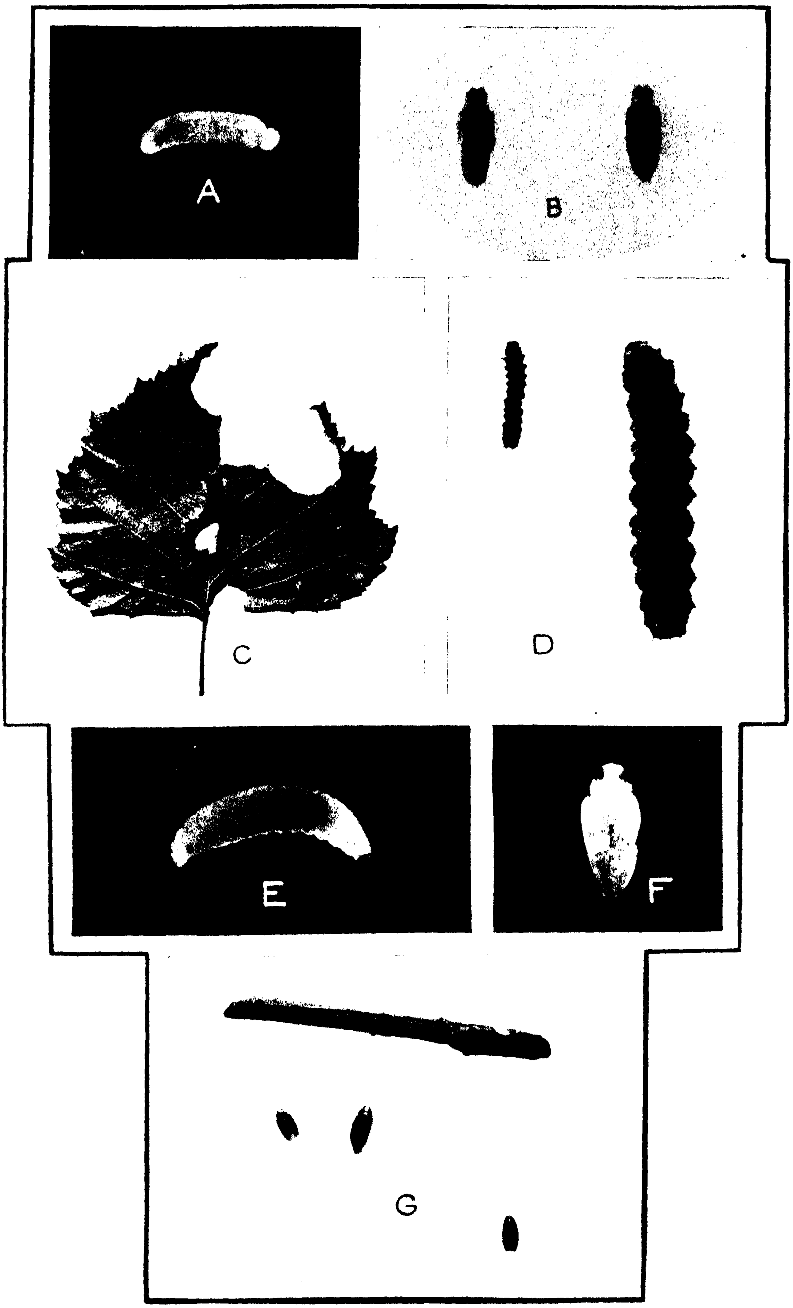
C.—Third-stage gipsy-moth caterpillar with cocoon of *A. lacteicolor.* Natural size.

D.—Two larvæ of an undetermined arctiid from the same egg mass: Above, parasitized by *A. lacteicolor;* below, unparasitized. ×2.

E.—*Meteorus versicolor:* Third-stage larva. ×5.

F.—*M. versicolor:* Pupa. ×5.

G.—*M. versicolor:* Cocoons. Natural size.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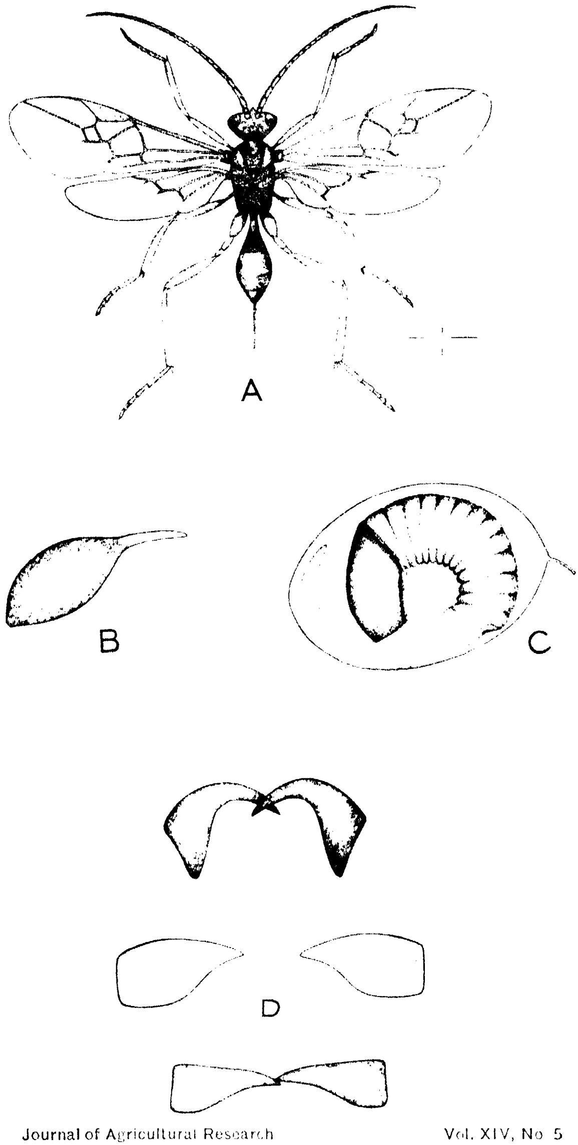
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PLATE 22

Meteorus versicolor:

A.—Adult female. Much enlarged.

B.—Egg. Much enlarged.

C.—Larva ready to issue from egg. Much enlarged.

D.—Larval mandibles: From top to bottom, first, second, and third stages. The mandibles of first and third stages are chitinized; those of the second stage not chitinized. Much enlarged.

A HITHERTO-UNREPORTED DISEASE OF OKRA

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INTRODUCTION

In the summer and fall of 1916 officials of the Office of Seed and Plant Introduction called the writer's attention to a disease of okra pods [*Abelmoschus esculentus* (L.) Moench.] found at the Yarrow (Maryland) Field Station of the Department of Agriculture. Later in the season material was brought in from the same place by Dr. B. T. Galloway, of the Bureau of Plant Industry, who reported considerable damage to the pods. In fact, the disease was so general and destructive that a comparatively small percentage of a full crop of healthy seed was harvested. The seed from the 1916 crop was not used either for distribution or for replanting, but a small amount of it was turned over to the writer for examination and experimental work.

The seed from which the diseased plants were grown had come originally from three different sources. One importation, SPI [1] 27810, came originally from Erivan, Caucasus, Russia, in 1910, and was planted at Yarrow for the first time in 1913 and was grown there each succeeding year up to and including 1916. While a part of the original seed was sent elsewhere to be grown, none of the seed grown at these other places was brought to Yarrow. Another importation, SPI 34165, from Lucknow, India, was received in 1912 and grown at Yarrow from 1913 to 1916. A third importation, SPI 41724, from Athens, Greece, was received in 1916 and grown there only one year (1916).

It is possible that this disease was imported with the seed from Russia, India, or Greece, since no reports of such a disease have been found from Maryland. That it can be carried on the seed is evident, from the fact that the causal fungus was isolated several times from seed collected from diseased pods, both before and after surface disinfection. In 1878 Cooke [2] describes a new species of Phoma, *Phoma okra*, on stems of okra collected by Ravenel in South Carolina. Similar material bearing a fungus attributed to the same species of Phoma was collected by Langlois in 1886 and again in 1887. The writer has examined Cooke's type material of *Phoma okra* as well as the specimens, and no septate spores were found.

In 1908 Barrus collected stems and pods of okra in the State of New York bearing a fungus which he identified as *Phoma okra* Cke. Some

[1] SPI=Office of Seed and Plant Introduction No.

[2] Cooke, M. C. NORTH AMERICAN FUNGI. *In* Hedwigia, Bd. 17, No. 3, p. 37–40. 1878.

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of this material was deposited in the pathological collections of the Bureau of Plant Industry, United States Department of Agriculture, and upon examination a large percentage of the spores were 1-septate. Spore measurements and other characters show it to be identical with the organism with which the writer has been working. This fungus therefore was present in this country before the importations mentioned above were made. In the light of these facts it is impossible to state definitely the source of infection. Either the fungus may have been imported with the seed or the infection of these plants may have originated from domestic sources.

DESCRIPTION OF PODSPOT

This disease has not been found to affect the leaves under natural conditions, and leaves sprayed with a suspension of the spores in water were not infected. Spots similar to those on the pods (Pl. 23, A) are found on the limbs (Pl. 23, B), but the damage there is relatively small. The greatest injury is done to the pods, and for that reason the common name "podspot" is proposed for this disease.

The causal organism grows rather slowly in the host tissue. There is little or no evidence of infection for a week or so after inoculation, but soon after that time a dark band, somewhat watersoaked in appearance, appears around the point of inoculation. From this time on development is a little more rapid, and a spot ½ to 1 inch in diameter results at the end of two or three weeks. Numerous pycnidia appear at about this time in the dead tissue. They continue to increase in number and to form in a more or less concentric manner as more host tissue is killed. On the death of the pod, pycnidia may or may not develop indiscriminately over the entire surface.

The spots are oval to oblong in shape, the longest diameter being the long way of the pod. The fungus follows the course of the fibrovascular bundles, and often the bundles may be found invaded and blackened some distance in advance of any evidence of the fungus on the surface. The fungus eventually grows through the pod, into and among the seed. Pycnidia were found on the seed, and the fungus was isolated from the seed after thoroughly washing and disinfecting the surface.

CAUSE OF OKRA PODSPOT

Isolations from diseased pods have always yielded a fungus bearing the characteristics of one of the Sphaeropsidaceae. Diseased pods and stems were wintered out with the hope that a perfect stage of the organisms might develop. However, even as late as June the same imperfect fungus was isolated from the old pods. Until more is known of the life history of the fungus it will be referred to the form genus Ascochyta (*A. abelmoschi*, n. sp.) and tentatively described as follows:

Ascochyta abelmoschi, n. sp.

Spots somewhat circular, often with a brown to black margin, more or less distinctly zonate; pycnidia gregarious, often crowded together, brown to black, lenticular, pyriform to globose, rather thick walled, at first buried, becoming finally erumpent, 65 to 225 μ in diameter, ostiolum small, mostly central; pycnospores, cylindrical to oval, straight or curved, 4.0 to 14.0 by 2.1 to 4.5 μ, hyalin, 1-celled for a long time, finally septating transversely at the center, then or not at all slightly constricted, rounded at the ends, when guttulate 2 to 4.

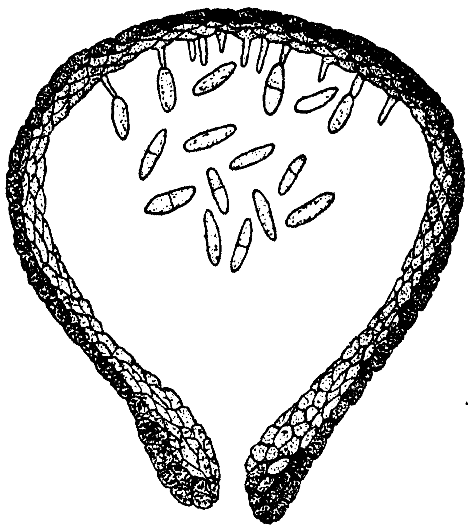


FIG. 1.—*Ascochyta abelmoschi:* A section through a pycnidium on the host showing the outer and inner walls, the sporophores and pycnospores. × 500.

Type specimens are deposited in the herbarium of the Pathological Collections of the Bureau of Plant Industry, United States Department of Agriculture.

While the pycnidia are relatively small when on the host, they are very much larger and more variable in shape when grown in artificial cultures.

The pycnidium is inclosed in an outer darkwall about one or two cells in thickness (fig. 1). Within this is a somewhat thicker hyalin layer from which the rather stout blunt conidiophores arise (fig. 2). The ostiolum arranged centrally on the host is variously placed when the fungus is grown in artificial cultures. It is small, slightly drawn out, and the pycnidial walls surrounding it are slightly thicker and darker than at other parts of the pycnidium.

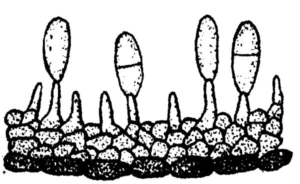


FIG. 2.—*Ascochyta abelmoschi:* A portion of the pycnidium shown in figure 1. × 1,000.

The spores (fig. 3), produced in great numbers, are hyalin and for a long time 1-celled. In old cultures and in the later stages of the development on the host some of the spores lay down a septum near the middle. Different specimens, as well as different cultures, vary as to the percentage of 2-celled spores, but no case has been found where more than 50 per cent of the spores have septated.

The spore may or may not be constricted at the septum. The spores of various forms, differing greatly in type, are straight or curved, and often larger at the ends than at the middle (fig. 3).

INOCULATION EXPERIMENTS

Podspot was reproduced with the characteristic symptoms on several varieties of okra and the organism recovered. It was again reproduced by inoculation from cultures resulting from such reisolations.

The original cultures were obtained in the fall of 1916, but the first inoculations were not made till some months later. Since okra does not grow well during the winter months under greenhouse conditions, plants were not started there till the spring of 1917. About August 1 abundant pods on these plants were suitable for inoculation. On August 10 a number were inoculated by inserting spores and hyphæ into wounds made with a dissecting needle. A few infections resulted from the inoculations, and the characteristic symptoms of the disease were produced. That a larger percentage of infection did not result was found later to be due to the fact that the pods were in most cases too mature when inoculated, young pods being much more susceptible. On August 15, pods in different stages of development, of five different varieties, New Lady Finger, Perkins Long Pod, Kleckleys Favorite, Dwarf Prolific, and White Velvet, were inoculated outdoors at the Arlington Experimental Farm as above with the same organism. Over 70 per cent of these pods developed the characteristic symptoms of the disease, the older pods remaining healthy or showing only slight infection. On August 20, pods, one-third to full grown but not mature, were inoculated in the greenhouse and 65 per cent became infected.

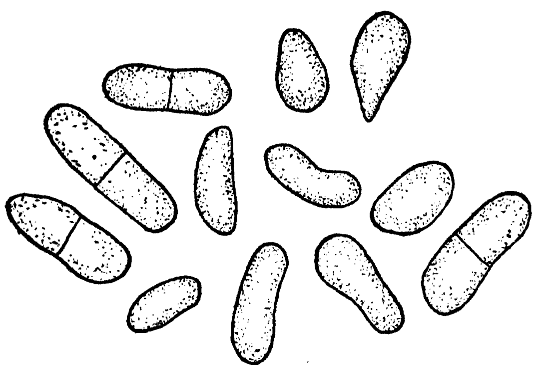


FIG. 3.—*Ascochyta abelmoschi*: A number of pycnospores, some of which are septated, showing the variations in shape and size. × 2,000.

The organism was recovered from the infected pods of the first inoculation and when fruiting abundantly was used in the greenhouse to inoculate pods in all stages of development. In some cases several pods on the same plant were inoculated. The results showed that the upper or younger pods were the first to show symptoms of the disease, and the spots enlarged more rapidly. The infected spot on the pod next below developed more slowly, while the pods lowest down on the stem either remained healthy or showed but slight evidence of disease and then only at the wound.

At the same time that this last pod experiment was carried out, leaves were sprayed with spores suspended in water, but no infection resulted. Since infected leaves were never found on diseased plants under field conditions, and spraying under artificial conditions yielded negative results, it is believed this is a disease of the pods and stems only.

Apparently several varieties of okra are susceptible to podspot. Infection takes place under natural conditions probably only when the pods are young, since old or nearly mature pods when wounded or inoculated usually resisted the fungus or showed but a slight indication of infection about the wound.

CULTURAL CHARACTERS

Ascochyta abelmoschi can readily be isolated in pure culture by the poured-plate method after a thorough washing of the infected pods, followed by surface disinfection in mercuric chlorid. It grows well on most of the agars, stems of *Melilotus alba*, cooked Irish potato cylinders, steamed rice and steamed corn meal. The maximum mycelial growth is made on agars and the minimum on Melilotus stems. On the other hand, it fruits sparsely on agars and abundantly on stems of *M. alba*. Growth is noticeable on most any of the media in common use in 24 to 48 hours at laboratory room temperature. On steamed rice and on steamed Irish potatoes an ocherous color appears in 48 hours and increases in intensity for several days. This color later gives way to a dirty ocherous color brought about largely by the development of the pycnidia.

Pycnidia develop not at all or sparingly on most of the agars. On stems of *Melilotus alba* they begin to appear in three days and to exhude spores in seven days. Pycnidia develop on rice and Irish potato cylinders in about three to four days and exhude spores in about eight days. Stems of *M. alba*, cooked rice, and Irish potato cylinders are good media for the growth of the fungus.

SUMMARY

(1) *Ascochyta abelmoschi* is parasitic upon the stems and pods of several varieties of okra.

(2) The disease caused considerable loss where it occurred in Maryland in 1916.

(3) The disease also occurs in New York State.

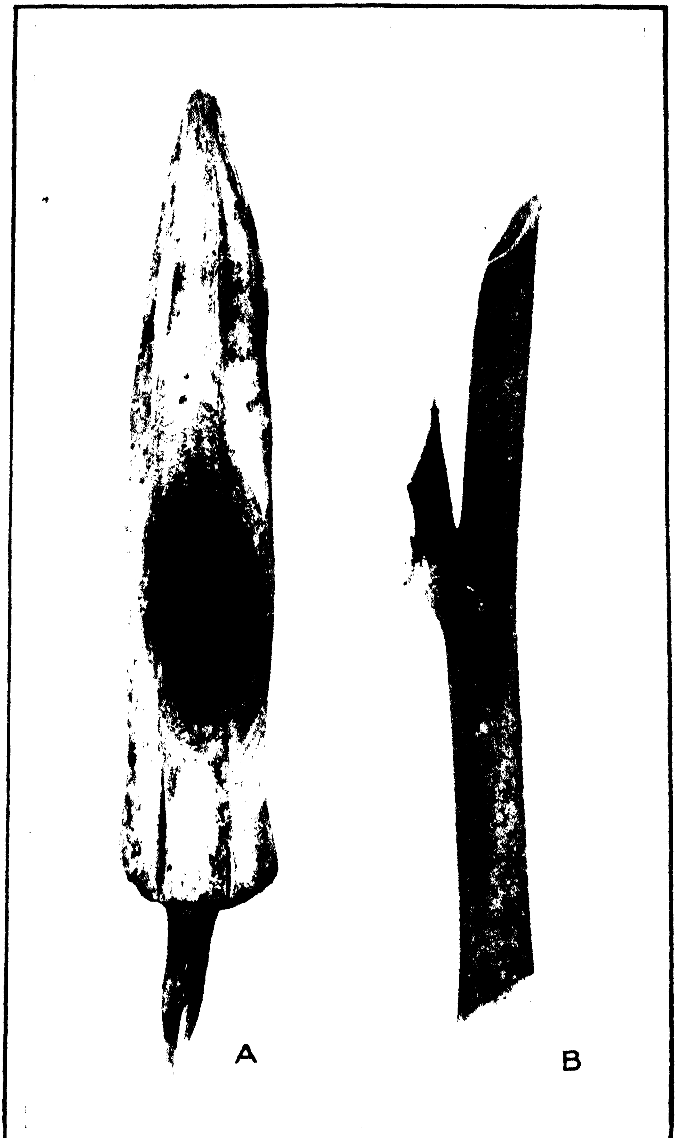
(4) The origin of this disease in Maryland is doubtful. Either it may have been imported with the seed or it may have originated from domestic sources.

(5) The fungus grows well on most any of the culture media in common use, but fruits the best on stems of *Melilotus alba* and cooked rice.

PLATE 23

A.—A pod of okra collected at Yarrow, Md., showing a typical spot caused by *Ascochyta abelmoschi*. The spots are usually more or less elliptical in shape and the pycnidia zonately arranged.

B.—A typical podspot infection on the stem of okra.


A
B

POTATO-STEM LESIONS

By H. A. Edson, *Pathologist*, and M. Shapovalov, *Agent, Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The brown canker-like areas occurring on the underground portions of the potato (*Solanum tuberosum*) plants in various parts of the country have been attributed by many writers to the attacks of *Rhizoctonia solani* Kühn. (Rolfs,[1] Morse, and Shapovalov,[2] Drayton,[3] and others.) On the other hand, Link[4] has found that similar lesions on potato stems in Nebraska may be due to species of Fusarium. Observations during the last few years, as well as numerous isolations and inoculation experiments, show quite conclusively that although Rhizoctonia and Fusarium may constitute the two principal genera of fungi responsible for the injuries in question, yet at the same time there are undoubtedly several other organisms hitherto not connected with this trouble which are capable of producing similar and even macroscopically identical stem lesions. The relative importance and frequency of the individual members of this group may vary throughout the country with the changing soil, season, and climate.

EXPERIMENTAL WORK

ISOLATIONS

A great number of isolations were made in the summers of 1916 and 1917 from the material collected on various farms in northern Maine. Both severely injured stems and stolons and those showing only small individual lesions served as material for this work. The majority of the cultures yielded *Rhizoctonia solani* and *Fusarium oxysporum*, then followed *F. discolor*, *Botrytis* sp., *Alternaria solani*, *Alternaria* sp., *Clonostachys* sp., *Acrostalagmus* sp., *Sclerotinia* sp. and a number of Hyphomycetes which failed to show parasitism in subsequent inoculation experiments. Different lesions on the same plant frequently yielded different fungi and, in several instances two parasites developed in cultures

[1] Rolfs, F. M. POTATO FAILURES. A PRELIMINARY REPORT. Colo. Agr. Exp. Sta. Bul. 70, 20 p., 12 pl. 1902.

——— POTATO FAILURES. A SECOND REPORT. Colo. Agr. Exp. Sta. Bul. 91, 33 p., 5 pl. 1904.

[2] Morse, W. J., and Shapovalov, M. THE RHIZOCTONIA DISEASE OF POTATOES. Maine Agr. Exp. Sta. Bul. 230, p. 193–216, fig. 61–73. 1914. Literature cited, p. 216.

[3] Drayton, F. L. THE RHIZOCTONIA LESIONS ON POTATO STEMS. *In* Phytopathology, v. 5, no. 1, p. 59–63, fig. 5, pl. 6. 1915. Literature cited, p. 63.

[4] Link, G. K. K. A PHYSIOLOGICAL STUDY OF TWO STRAINS OF FUSARIUM IN THEIR CAUSAL RELATION TO TUBER ROT AND WILT OF POTATO. *In* Bot. Gaz., v. 62, no. 3, p. 169–209, 13 fig. 1916. Literature cited, p. 207–209.

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from a given lesion. In the spring of 1918 several specimens were obtained from Florida, some of which had characteristic brown lesions on the stems. Plantings made from these diseased areas yielded pure cultures of *F. oxysporum.* Identical results were also obtained with some of the material collected recently in the vicinity of the District of Columbia.

INOCULATIONS

Although certain preliminary tests were made in the field, this work was carried on chiefly under greenhouse conditions at Washington, D. C., with disinfected seed and steam-sterilized soil and pots, watered from the city mains supplied with clarified and filtered Potomac River water. The following method of inoculation has been found very satisfactory: When the young plants were about 2 or 3 inches high, the soil was removed at one side of the plant clear down to the seed piece (on the average, about 2 inches from the surface of the soil), care being taken to avoid as much as possible any injury to the epidermis of the stem. Then the culture was removed from the test tube on a piece of clean absorbent cotton, and the preparation was placed on the exposed side of the stem. Each fungus had been grown for this purpose on three kinds of medium; rice, cooked potato cylinders, and melilotus stems. Finally the soil was replaced and heaped up about the plant, nearly covering it. Control plants were treated in a similar way except that sterile culture medium instead of fungus cultures was applied to the stems. About four weeks after inoculation the plants were carefully dug, washed, and examined. Besides the fungi isolated from the potato, a number of Rhizoctonia strains[1] from various other hosts, as well as authentic cultures of several species of Fusarium, were included in the tests. The combined detailed results of these experiments are given in Table I.

TABLE I.—*Results of the potato-stem inoculations with Rhizoctonia solani, Fusarium spp., and several other fungi*

Name of culture.	Date of isolation.	Number of plants inoculated.	Number of plants infected.	Character of injury produced.
Rhizoctonia solani (*R. potomacensis* Wollenw. from tomato).	August, 1912...	8	8	Irregular, deep, dark-brown lesions on stems; stolons and new tubers also affected.
R. solani, R. Kan. (from beets).	1913..........	5	3	Brown discoloration at the base of the stems.
R. solani, R. S. (from beet seedlings).	July, 1912.....	5	3	Large brown canker in one case and a slight russetting in two others.

[1] The word "strain" in its application to Rhizoctonia is used in the present paper merely to differentiate cultures obtained from different hosts or from the same host from different localities. It does not imply any reference to their taxonomic relationships.

TABLE I.—*Results of the potato-stem inoculations with Rhizoctonia solani, Fusarium spp., and several other fungi*—Continued

Name of culture.	Date of isolation.	Number of plants inoculated.	Number of plants infected.	Character of injury produced.
R. solani, Hyp. I (from potato).	October, 1914..	8	5	A number of medium-sized deep and very dark cankers on stems.
R. solani, R. V (from beet seedlings).	September, 1911.	5	0	....
R. solani, R. VI (from beet seedlings).	.....do........	8	8	Large, deep, irregular, medium-dark necrotic areas on stems.
R. solani, R. VII (from beets).	October, 1911..	6	6	Numerous small dark-brown spots on stems; brown discoloration on roots present.
R. solani, R. XI (from potato).	October, 1912..	8	8	Dark-brown cankers on stems and stolons.
R. solani, R. XII (from beets).	September, 1912.	8	6	Small, light-brown spots on stems.
R. sp., R. XIII (from onion).	May, 1911......	3	0	....
R. solani, R. XIV (from radish).	February, 1913.	3	0	....
R. solani, R. XV (from pine seedlings).	June, 1911.....	6	6	Small lesions on stems and some on stolons.
R. solani, R. XVI (from beets).	1912..........	5	2	Small brown spots on stems.
R. solani, R. XVII (from peanut).	1914..........	5	3	Do.
R. solani, R. XVIII (from potato).	October, 1914..	3	3	Do.
R. solani, R. XX (from potato).	1914..........	3	0	....
R. solani, R. XXIII (from alfalfa).	September, 1914.	3	3	Small dark-brown spots on stems; younger shoots and some of the roots killed.
R. solani, R. XXIV (from potato).	June, 1915.....	3	1	Large necrotic area on one stem.
R. solani, R. XXV (from carnation).	September, 1915.	5	2	Brown spots on stems.
R. solani, R. XXVI (received from Amsterdam).	1916..........	3	2	Large irregular lesions on stems; some infection on stolons present.
R. solani, R. XXVII (from potato.)	1916..........	3	0	....
R. solani, R. XXIX (from potato).	July, 1916.....	4	2	Brown lesions on stems.
R. solani, R. XXX (from potato).	July, 1916.....	4	3	Deep cankers on stems.
R. solani, R. 724 F (from pine).	1916..........	3	3	Pronounced depressed brown lesions; especially severe on stem inoculated with rice culture.
R. solani, R. 147 W (from spruce).	1910..........	3	3	Distinct but small and shallow spots on stems; more at the crown.
R. solani, R. 187 K (from potato).	1910..........	3	3	Only slight browning on stems.

TABLE I.—*Results of the potato-stem inoculations with Rhizoctonia solani, Fusarium spp., and several other fungi*—Continued

Name of culture.	Date of isolation.	Number of plants inoculated.	Number of plants infected.	Character of injury produced.
R. solani, R 186 L (from potato).	1910	3	2	No infection in case of melilotus culture and only slight browning on stems inoculated with rice and potato cultures.
R. solani, R. 361 L (from pine.	1915	3	0	....
Fusarium coeruleum	March, 1915	3	3	Small dark lesions on stems, stolons, and roots.
F. discolor	1908	8	8	Lesions on stems and occasionally on roots; in two instances injury very severe (from rice culture), others only slight.
F. discolor var. *sulphureum*	June, 1909	3	3	Small lesions on stems and stolons.
F. eumartii	January, 1914	6	6	Serious stemrot in two instances; deep cankers on the remaining stems; lesions on roots and stolons.
F. oxysporum	October, 1916	8	8	Deep irregular, brown lesions on stems and occasionally on stolons.
F. radicicola	February, 1916	4	3	Deep dark-brown lesions on stems, stolons, and roots (melilotus culture produced no effect).
F. solani	February, 1914	3	0	Only slight discoloration.
F. trichothecioides	October, 1916	4	4	Very large dark, deep cankers on stems; lesions on on roots and stolons.
Alternaria sp. I	1916	5	5	Distinct brown necrotic areas varying in severity on different plants.
A. solani	1916	3	3	Large dark-brown cankers on stems.
Botrytis sp. I (from potato leaf).	1916	8	8	Distinct brown but shallow lesions on stems.
Botrytis sp. II (from potato stem).	1916	6	6	Severe dark-brown lesions on stems.
Sclerotinia sp. (from a sclerotium inside of stem).	1916	2	2	Do.
Zygorhynchus sp	1917	3	3	Shallow lesions on stems and stolons.
Corethropsis sp	1917	3	3	Shallow lesions on stems.
Phoma sp	1916	6	4	Superficial russeting only (on stems).
Acrostalagmus sp	1917	6	6	Irregular, brown, lesions on stems.
Clonostachys sp	1917	6	6	Irregular, light-brown, somewhat depressed lesions on stems.
Verticillium albo-atrum I (from eggplant).	September, 1915	3	3	Slight amount of russetting on stems.
V. albo-atrum (from potato).	1917	3	3	Slight discoloration of stems.
Verticillium sp. (from okra).	1917	3	3	Do.

In addition to the fungi listed above a miscellaneous group of apparently saprophytic organisms obtained in cultures made from potato stems was included. This group comprised species of Penicillium, Phoma, Chaetomium, and several unidentified fungi. Triplicate inoculations were made with each member of the group. In a few instances a faint brownish discoloration was observed on stems just beneath the cotton covering the inoculum; however, its amount was too insignificant to warrant a conclusion that any of these organisms were pathogenic. The most serious infection was secured with several strains of *Rhizoctonia solani*, *Fusarium eumartii*, *F. oxysporum*, *F. radicicola*, *F. trichothecioides*, *F. discolor*, *Alternaria solani*, *Botrytis* sp. from potato stem, *Sclerotinia* sp. from potato stem, *Acrostalagmus* sp., and *Clonostachys* sp. The character of injury produced by various fungi, as well as the appearance of the control plants and those inoculated with saprophytic species, is illustrated in Plates 24, 25, and 26. Control plants remained free from underground injuries with the exception of a few stems, when a slight browning such as was noted in case of some saprophytes was present (Pl. 26, I–L).[1]

The degree of parasitism of the different strains of Rhizoctonia varied from absolute absence of any visible injury to the formation of large and deep cankers. This phenomenon has been already noted by Rosenbaum and Shapovalov [2] with regard to their strains of this fungus. In the present work the evidence was even more striking. Not only the size and the depth of the lesions were unlike, but also their color and shape were quite peculiar to certain particular strains. Moreover, these characters were not incidental to one series only, but, on the contrary, with certain strains quite constant in every series of inoculation. For example, *Rhizoctonia potomacensis* Wollenw. always produced dark-brown and deep lesions, and similar injury resulted from R. XI and from R. 724 F, while R. VI invariably formed large, deep and medium-dark necrotic areas; R. VII and R. XXIII produced small dark spots, R. XII and R. 147 W small light-brown spots, and R. V., R. XIV, R. XX, R. XXVII, and R. 361 L in no case produced any injury whatever. With the remaining strains of Rhizoctonia the peculiarities were not so constant nor so distinct.

The virulence of the different strains did not appear to be correlated in any way with the length of time they had been carried in artificial culture or with the host from which they were originally isolated. Thus R. V and R. VI, isolated from sugar-beet seedlings in September, 1911, produced respectively, no injury and large, deep, irregular necrotic

[1] Certain greenhouse experiments conducted since the completion of this work indicate that *Penicillium oxalicum* also is able to produce distinct brown lesions on the potato stems inoculated with pure cultures of this fungus.

[2] ROSENBAUM, J., and SHAPOVALOV, M. A NEW STRAIN OF RHIZOCTONIA SOLANI ON THE POTATO. *In* Jour. Agr. Research, v. 9, no. 12, p. 413–419, 3 fig., pl. 25–26. 1917.

areas. R. XI, isolated from potato in October, 1912, R. XII, isolated from beets in September, 1912, R. XV, isolated from pine seedlings in June, 1911, and R. XXIII, isolated from alfalfa in September, 1914, uniformly yielded positive results, while R. XXIX and R. XXX, isolated from potato in July, 1916, gave positive results only in two out of four and three out of four cases, respectively, and R. XX, isolated from potato in 1914, and R. XXVII, isolated from potato in Europe and received from Amsterdam in 1916, were among the strains constantly giving negative results. Two other European strains employed, Hyp. I and R. XVIII, isolated from potato October, 1914, gave positive results five times out of eight and three times out of three, respectively. These two strains were isolated and contributed by Dr. Pethybridge, who stated that Hyp. I was obtained from a single spore of *Hypochnus solani* (*Corticium vagum*) developing on potato stems in Ireland. *Rhizoctonia potomacensis* Wollenw., which appears to differ in no way from *Corticium vagum* B. and C. and which was isolated from tomato in September, 1912, was one of the most aggressive strains employed. It was strongly parasitic on tomatoes and on sugar beets both as a damping-off fungus and in the production of rootrot of adult plants. Five strains were contributed by Mr. Carl Hartley, of the Bureau of Plant Industry, with data regarding their origin and virulence as damping-off agents on pine seedlings. Named in the order of diminishing virulence on potato stems they are R. 724 F from pine, strong but not maximum virulence on pine; R. 147 W from spruce, maximum virulence on pine; R. 187 K from potato, moderate to weak virulence on pine; R. 186 L from potato, nearly or entirely nonparasitic on pine; R. 361 L from pine, moderate to weak virulence on pine. The two strains most active on pine were also most virulent to potato stems but in reverse order; R. 187 K was mildly pathogenic to both hosts; while R. 361 L, mildly parasitic on pine, did not injure potato stems, and R. 186 L, nonparasitic to pines, was only very mildly pathogenic to stems.

Certain variation in the amount of infection was noted in connection with the viability of a particular organism in pure culture. Thus, for instance, as a rule, little injury, or none, resulted from inoculation with Rhizoctonia cultures grown on melilotus stems, which is not a very satisfactory medium for this fungus, while rice and potato cultures produced severe lesions.

The infection with *Fusarium eumartii*, *F. radicicola*, *F. trichothecioides*, both species of Alternaria, *Botrytis* sp. from stem, and *Sclerotinia* sp. from stem as a rule produced deep necrotic lesions, sometimes taking on the appearance of a dry stemrot. This was especially true with *F. eumartii* (Pl. 25, C–I). *F. oxysporum*, which in these trials did not penetrate the vascular elements, showed a distinct ability to attack violently other tissues of the potato stem. On the other hand, *F. trichothecioides*, after

penetrating the stem to a considerable depth, invaded the vascular bundles in one instance. Subsequently dark-brown discoloration developed and the organism was recovered in pure cultures from the petioles of the topmost leaves.

CONCLUSIONS

(1) Neither *Rhizoctonia solani* Kühn nor any particular species of Fusarium can be held as the sole agents responsible for the familiar stem and stolon lesions of the potato.

(2) Several parasitic species of Fusarium, as well as Alternaria, Botrytis Sclerotinia, Zygorhynchus, Corethropsis, Phoma, Clonostachys, Acrostalagmus, and probably other fungi, should be included with certain strains of Rhizoctonia in the group of the causal organisms.

(3) While this group of parasites may be quite large, a number of strains of Rhizoctonia, along with other saprophytic species, are associated with stem lesions which are unable to attack the underground portions of the potato plant.

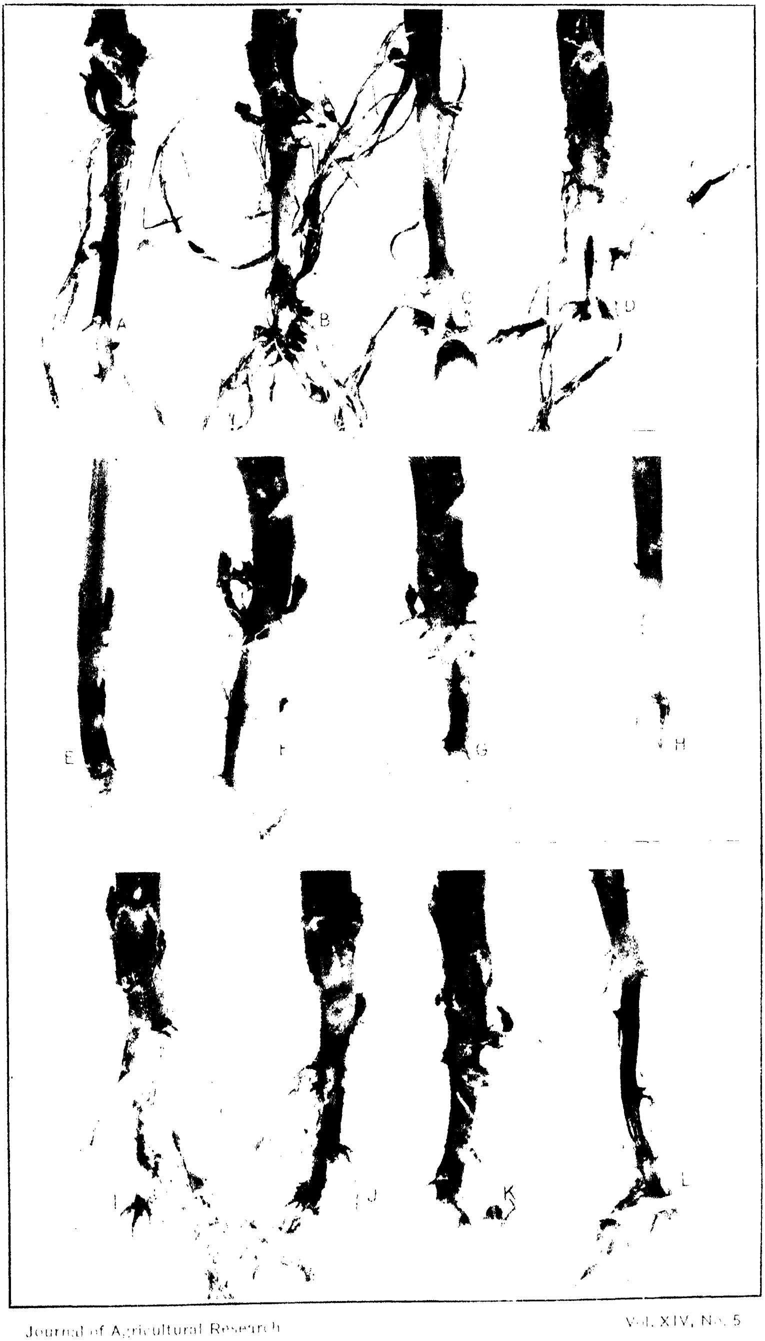
(4) The lesions produced by some of these fungi, while practically inseparable by their macroscopic characters when produced under natural field conditions, show distinct characteristics peculiar to certain strains or species when reproduced under control conditions in the greenhouse.

PLATE 24

Potato-stem lesions four weeks after inoculation:

A–H, Plants inoculated with *Rhizoctonia solani:* A, R. VII; B, C, E, *R. potomacensis;* D, Hyp. I; F, R. S.; G, R. VI; H, R. XVI.

I–L, Plants inoculated with species of Fusarium: I, *F. solani;* J, K, *F. radicicola;* L, *F. trichothecioides.*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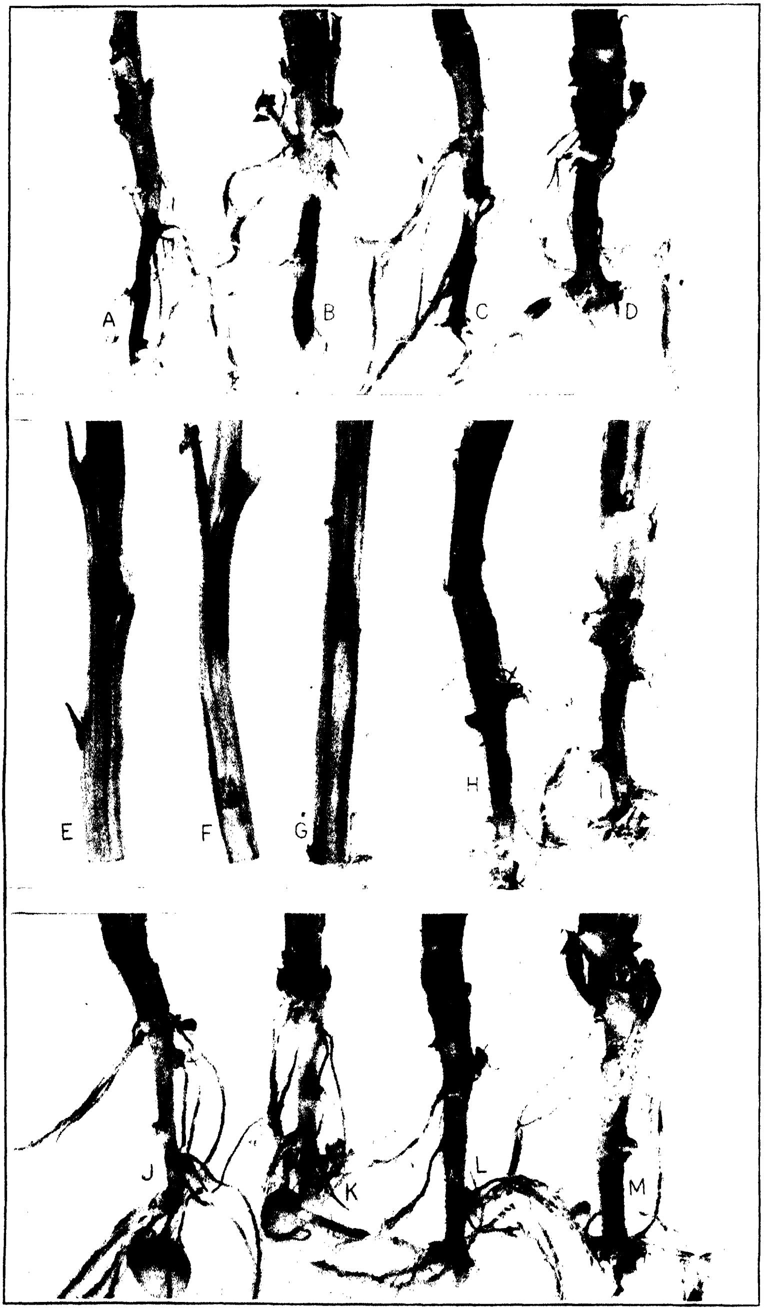
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PLATE 25

Potato-stem lesions four weeks after inoculation:

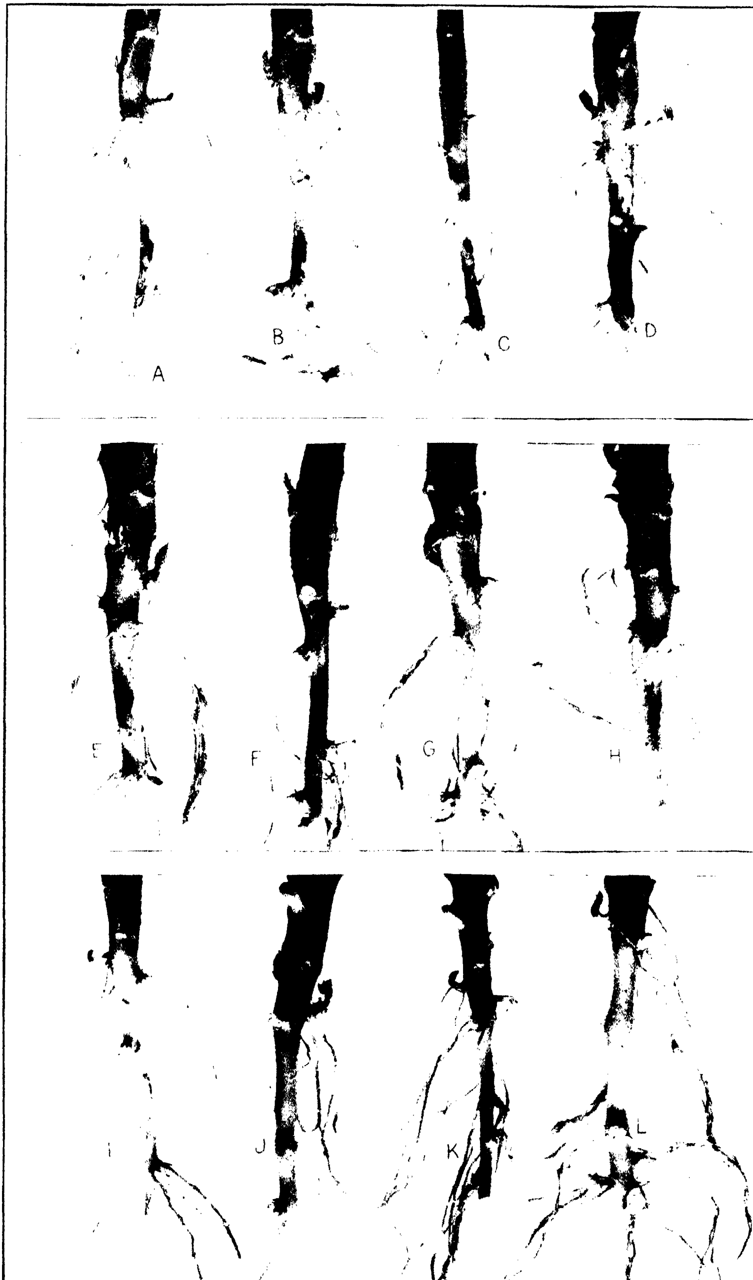
A–L, Plants inoculated with species of Fusarium: A, *F. discolor;* B, *F. oxysporum;* C–I, *F. eumartii;* E–H represent portions of the same plant, showing necrotic areas throughout the stem; J, *F. coeruleum;* K, *F. solani;* L, *F. discolor* var. *sulphureum.*

M, Plant inoculated with *Botrytis* sp. I.

PLATE 26

Potato-stem lesions four weeks after inoculation with miscellaneous fungi:

A, *Clonostachys* sp.;
B, *Zygorhynchus* sp.;
C, *Alternaria* sp. I;
D, *Alternaria* (*Macrosporium*) *solani*;
E, *Phoma* sp.;
F, *Corethropsis* sp.;
G, *Chaetomium* sp.;
H, *Acrostalagmus* sp.;
I–L, Control plants.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ANATOMY OF THE POTATO PLANT, WITH SPECIAL REFERENCE TO THE ONTOGENY OF THE VASCULAR SYSTEM[1]

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COOPERATIVE INVESTIGATIONS BETWEEN THE BUREAU OF PLANT INDUSTRY OF THE UNITED STATES DEPARTMENT OF AGRICULTURE AND THE AGRICULTURAL EXPERIMENT STATION AT CORNELL UNIVERSITY

INTRODUCTION

Recent studies of potato diseases have made it obvious that more accurate knowledge regarding the normal structure of potato plants (*Solanum tuberosum*) is necessary. The present work was undertaken in order to obtain data which may make possible a more rapid progress in the investigation of several important potato diseases, such as the potato leafroll. This disease, which is a serious one, was the main reason for undertaking the present detail study. It was the original intention of the writer to investigate the pathological anatomy of plants suffering from leafroll, but because of the lack of adequate information regarding their normal structure no information for comparative purposes was available.

The anatomy and development of the potato plant have been studied up to this time chiefly from the viewpoint of economic botany; consequently previous work relates largely to observations on gross morphology. A study of the origin, differentiation, and organization of the vascular supply, however, is essential to a clear understanding of the physiology of the organs and of the relation of this supply to tuber formation. Furthermore, such a study enables us to distinguish the significant points in all pathological changes.

The critical study of the internal anatomy of plants began only in the middle of the last century, when the work of Schleiden and other investigators raised botany to the level of other sciences. Schleiden, in his

[1] This work was begun in the field laboratory of the Office of Cotton, Truck, and Forage Crop Disease Investigations at Greeley, Colo., in the summer of 1916, and was continued in the Department of Plant Pathology at Cornell University under the direction of Prof. H. H. Whetzel and Dr. H. A. Edson, to whom the author wishes to express his gratitude for their courtesy and helpful suggestions. To Dr. A. J. Eames, of the Department of Botany, Cornell University, the writer is especially indebted for the constant advice and criticism received in preparation of materials, interpretation of slides, and editing of the paper. Thanks are also due to Mr. W. R. Fisher for accurate and painstaking work in the preparation of the photographs.

"Cell Theory," clearly set forth the relation between cells, tissues, and organs, and also treated in some detail the origin and function of each. Other investigators, stimulated by his work, added new facts and corrected the old errors, until finally a fairly clear understanding of the general internal anatomy of plants was gained. As may be expected, much of this earlier work relates to the xylem, as this tissue is by far the most easily distinguished under low magnification. The phloem remained a mystery up to the time of Hartig. This investigator reports and describes phloem elements, but it remained for Von Mohl to give a clear conception of the sieve tube and to show its significance as a conducting unit for plastic materials. Von Mohl, and later other investigators, reported the occurrence of sieve tubes in numerous plant families, but Hanstein (*2*)[1] was first to report their occurrence in the Solanaceae, the family to which the potato belongs.

Vesque (*5*) gives a short discussion on the distribution of the external and internal phloem in the Solanaceae. He also reports the occurrence of phloem fibers, but states that they are absent in the internal phloem when they are wanting in the external region.

Petersen (*9*) confirms Vesque and slightly extends his observations on the distribution and the relative amount of external and internal phloem in the different genera of the Solanaceae.

The first detailed discussion of the histology of the Solanaceae is given by Weiss (*10*). He writes as follows:

> The internal phloem groups always accompany the leaf-traces into the leaf and are differentiated only a little earlier than are the groups of external phloem. A distinct cambium is never developed between internal phloem and xylem. The internal phloem groups remain distinct and only in the smaller veins of the leaf blade do they unite with the external phloem. On the other hand, the internal phloem may be considered as derived from the external phloem, a theory which would explain the presence in the pith of fibers characteristic of the external phloem.

In 1872 Jurgens (*4*) published a thesis on the anatomy and physiology of the potato tuber in which he gives a general yet comprehensive account of the histological structure and development of the tuber as well as of the plant itself. Unlike other investigators of his time, he did not believe that the periderm is formed from the epidermis, but from the subepidermal layer, the original epidermis having become sloughed off.

The work of Schacht (*1*) is of interest only on account of its numerous and beautiful plates illustrating the internal anatomy and the morphology of the plant and the tuber.

De Vries, after publishing two papers, one on the development and germination of the potato tuber (*7*), the other on the seed (*6*), reported his researches on the anatomy and the physiology of the potato plant in the "Landwirtschaftliche Jahrbücher" (*8*). This paper is of considerable importance, especially for the physiologists. In fact, it is the only

[1] Reference is made by number (italic) to "Literature cited," pp. 251–252.

paper treating of the physiology of the potato plant in detail and in all aspects. The gross morphology, the anatomy of the organs and tissues, as well as the development of the leaf, are given careful consideration. However, valuable as is this paper, the author tells us nothing definite of the ontogeny of the vascular system or of the relative amount and relations of the different elements of the phloem and xylem. Moreover, since this work was written, our conception of the origin of the stele and of its relation to the leaf traces has undergone a radical change, so that, so far as histology is concerned, the work is out of harmony with present ideas. This deficiency in De Vries's work and the absolute lack of other reliable study make an investigation of the anatomy of the potato plant imperative, the more so since the recent studies of potato diseases require a clear conception of normal structures as a background for the investigation of the changes brought about by pathological conditions. With the purpose of meeting this need, the present study was undertaken. Here an attempt is made to clear up the points left in doubt by earlier workers. In addition new facts are given which may make possible a decision between the divergent views of present experimenters who have been working on the physiological importance of pathological changes. Several potato diseases have been studied with reference to such changes but of special interest and importance at the present time is the leafroll disease, which is causing a serious loss both in this country and in Europe. A discussion of these divergent views, together with new investigations on the subject of the leafroll disease, will be given in a later paper; the present contribution is concerned with the anatomy of the normal plant.

MATERIAL AND METHODS OF EXPERIMENTATION

The material for study was obtained both from plants grown in the greenhouse and in the disease garden of the Department of Plant Pathology of Cornell University. The Irish Cobbler variety furnished the material for investigation; other varieties, such as Early Rose, New York Rural, and Green Mountain were used for comparative study. Several fixing fluids were used, including those of Flemming; but the best results were obtained by the common chromacetic-acid fixer, 1 per cent chromic acid and 1 per cent acetic acid. The usual methods of dehydrating and embedding in paraffin were employed. Leaf sections were cut 5 μ thick, stem sections 7 to 12 μ thick, and stained with Haidenhain's iron alum hematoxylin and safranin.

GROSS MORPHOLOGY

Solanum tuberosum L. is an annual herbaceous dicotyledon of the Solanaceae. In habit it is more or less spreading, bu height of 2 to 5 feet. This habit form is constant, tho culture, and breeding have often brought about so

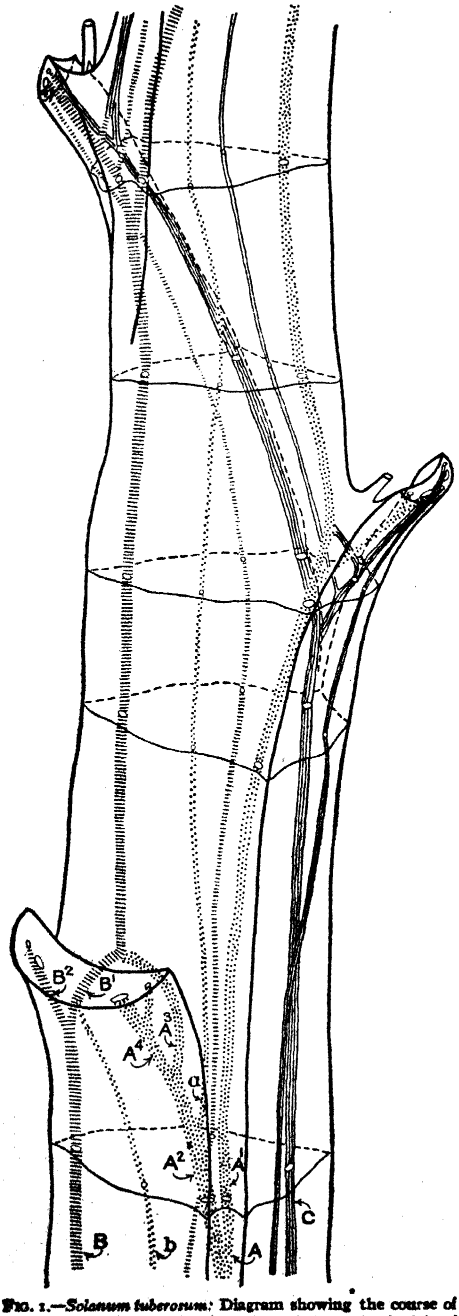

FIG. 1.—*Solanum tuberosum:* Diagram showing the course of the vascular bundles in the stem and the mode of origin of the vascular supply of the leaves (longitudinal). (See p. 227.)

The vascular cylinder is not of uniform width throughout; projections into the pith occur in places (Pl. 41, B). Its bundles are in part free, in part united by interfascicular cambium. The three corners of the stem are occupied each by a large bundle, and between each two of these a smaller bundle is found (Pl. 27, A). The vascular tissue follows naturally a longitudinal course in the stem, and its arrangement is closely related to the scheme of phyllotaxis. In traversing the stem in a vertical direction these bundles always remain approximately at the same distance from the center, entering the leaf as a whole or in part. Those bundles or strands of vascular tissue which pass out into a leaf are known as leaf-trace bundles. The relation of these to the stem is of much importance, since each leaf trace not only supplies the leaf to which it belongs with water and minerals, but also translocates the plastic materials manufactured in the leaf. The diagram (text figure 1) and the figures of Plates 27 and 28 show the course and origin of the various traces and their relation to each other and to the stem. A study of the petiole of the leaf shows that the vascular tissue, which occupies a semicircular area, consists of five groups: Three large groups and two smaller ones, the latter lying

in the outer corners (Pl. 43, C). These five groups are distinct traces, and their origin must be established and their course followed. A series of cross sections through as many internodes as are traversed by the longest trace is required for this task. Since the scheme of phyllotaxis has a divergence of 5/13, it may be supposed that the greatest extent of any of these traces would not exceed three internodes; this is actually the case.

As stated above, there are three large and three small stem bundles in the internodal region (Pl. 27, A). Below the node (base of diagram, figure 1, which illustrates a right spiral), where the stem begins to lose its three-cornered appearance and takes on the quadrangular outline, main group A begins to widen and breaks up into two more or less equal portions, A^1 and A^2. A little higher up, main group B also begins to widen. Immediately below the node, A^2 gives off a small bundle, a, which soon separates from the parent group and comes to lie between A^1 and A^2. (See also Pl. 27, B, a.) Just at the node, groups B and A^2 split, each forming two groups of nearly equal size: B gives rise to B^1 and B^2, and A^2 gives rise to A^3 and A^4. B^2 and A^4, given off by B and A^2, respectively, then divide unequally once more. These four last-mentioned groups pass out into the petiole forming the lateral traces there. The small stem group b, occupying originally the position between A and B, also passes out, but without division, into the petiole where it forms the median trace of the leaf. We see then that the vascular tissue of the petiole is derived from two chief sources:

(1) One of the smaller bundles of the stem, which becomes the median trace of the leaf.

(2) Branches of the two large stem groups A and B, which form the four lateral traces. The traces of the petiolar wings are derived from the lateral groups B^2 and A^4 after these have become separated from the stem bundles.

At the place of first branching of A, A^1 continues the original course of A. At the place of second branching those portions (B^1 and A^3) of B and A^2 which do not enter the petiole unite just above the insertion of the leaf to form a new stem bundle. Through this fusion the stem becomes three-cornered again.

The stem group A supplies two lateral traces of the petiole, gives rise to a new stem group A^1, and also to a new median trace, a, which becomes the median trace of the third leaf above; it further gives off some of its vascular tissue to B^1. B also supplies two lateral traces to the petiole, but does not form a third bundle; on the contrary, it receives vascular tissue from A^2 in compensation for the loss.

Bundle A^1 ascends for one internode and then forks again, but B^1 ascends unchanged for two internodes, then forks and gives rise to the same number of groups as B gave off near the first node, becoming the B of the second node. Bundle C similarly becomes the A of the second

node. This behavior of the bundles continues uniformly at succeeding nodes.

To summarize:

(1) The median trace ascends without fusion or forking through three internodes, and then passes into the petiole without branching.

(2) The lateral traces are given off at the node from two of the three large stem groups.

(3) Each large stem bundle ascends without branching in turn for two internodes and for one internode.

(4) Where a large stem bundle ascends for but one internode without branching, it divides three times, giving rise upon the first division to a new stem bundle, upon the second division to a new median trace, and upon the third division to lateral traces of the petiole. Upon the division in the node, half of the tissue given off to unite with that from the left (in a right spiral) adjacent group, forming a new large bundle directly above the insertion of the leaf. Where the bundle ascends for two internodes without branching, it gives off vascular tissue only for lateral traces of the petiole.

(5) Each leaf derives its supply from two large bundles and the smaller one lying between these. The method of derivation is uniform for all leaves, the bundles taking part being each time a different pair from those supplying the leaf below. In a right spiral the right member of the set supplying a leaf supplies also the leaf above, becoming there the left member of the set. The median trace of a given leaf is formed just below the third node below the leaf it supplies.

The vascular tissue shows bicollateral arrangement of its elements, a condition most clearly seen in the larger bundles of the stem. An examination of Plate 29, A, shows at first glance a wedge-shaped mass of rather large, dark-staining cells, the xylem. At more or less equal distances to the outside and the inside of the xylem are small groups of thin-walled cells which make up the phloem. The external phloem is separated from the xylem by a layer of uniform, rectangular cells, the cambium. The internal phloem is also separated from the xylem, but in this case by thin-walled, irregular cells (Pl. 29, C). These cells are much smaller than those of the pith and form what may be called the perimedullary zone of the vascular cylinder (stele). The external phloem groups are separated from one another and from the endodermis by parenchymatous cells of irregular size, which together constitute the pericyclic region of the stele (Pl. 29, A, B). The internal phloem abuts directly on the pith, and many of its groups are completely surrounded by pith cells. The phloem in both regions is made up of cell groups which in the outer zone are small and form a more or less continuous band and which in the inner region are variable in size and more scattered (Pl. 29, C).

A close examination of the xylem (Pl. 42, A) shows that the larger outer cells are arranged somewhat in radial rows, in which the individual cells either abut on one another or are separated by small thin-walled parenchyma cells. The walls of these elements are scalariform, reticulate, or pitted (Pl. 32, C; 33, E). Occupying the tip of the wedge-shaped mass are smaller xylem elements scattered among parenchyma cells. These have secondary thickenings in the form of rings or spirals and are the elements which mature before elongation ceases, the so-called protoxylem (Pl. 29, A, C; 42, C). Frequently these protoxylem elements appear crushed (Pl. 29, C), and show only part of the secondary thickening of the wall—that is, half a ring or part of a spiral band. The larger elements external to these cells make up the metaxylem. Though the arrangement of the xylem cells is somewhat irregular, the smallest, oldest, elements are found adjacent to the pith, showing that the order of development is from within out or centrifugal, and that the arrangement of these cells is therefore endarch.

At this stage both the xylem and the phloem are entirely primary—that is, of procambial origin. The phloem consists of small groups of sieve tubes with their companion cells and thin-walled conducting parenchyma (Pl. 31, A, B). Phloem fibers are first observed in slightly older tissue. When present, they occur singly or in small groups on the inner face of the endodermis, and in the outer region of the pith. Groups of primary phloem occur not only to the inside and outside of the primary xylem groups but are equally prominent in the interfascicular region where they may be seen at varying distances on both sides of the well-developed interfascicular cambium (Pl. 29, A). The outer phloem groups are small and close to the cambium; the inner are larger and more distant.

Since the structure and development of the phloem are the principal objects of this study, the cells of the xylem are considered only briefly. The vessels are porous, and of the type usually found in herbaceous angiosperms. The vertical extent of the individual cell is about two to three times its diameter. The end walls are usually somewhat oblique (Pl. 33, E), but sometimes nearly transverse (Pl. 32, B). As is usual in vessels, the walls are heavily pitted, the pits being arranged in transverse series (Pl. 33, E). Typical tracheids and wood parenchyma cells are found scattered among the larger vessels. In places these parenchyma cells are arranged in radial rows, forming narrow bands one or two cells wide. These are the innermost cells of the first-formed medullary rays, those extending from the pith itself, rays which may be called "primary medullary rays." In stained sections these cells are distinguished not only by their arrangement, regular size, and shorter tangential diameter but also by their darker stain. The protoxylem elements have already been considered and will be described in detail in the study of the ontogeny.

Just as the vessel is the most important element of the xylem, so is the sieve tube the principal and most interesting element of the phloem. In the potato the tubes have a cylindrical shape, with end walls strictly transverse. A single sieve plate occupies nearly the entire transverse wall, and no plates are found in the radial and tangential walls. The plate appears to be perforated by a large number of circular pores, as is seen clearly in Plate 33, B, and in the enlarged view (Pl. 33, D). Sieve fields as such do not exist in this plant; each large pore of the plate represents the sieve field of forms with primitive phloem. There is little variation in the size of the sieve tube in both inner and outer phloem. The length of the individual sieve-tube segment is, on the average, 138 μ; the diameter of the lumen varies from 17 to 32 μ. Whenever branching of the bundle and anastomosis occurs, the size of the sieve tube varies much more (Pl. 34, A, B). At such places the elements are short and comparatively broad (Pl. 35, A, B). When the sieve-tube mother cells undergo division, the larger of the two cells formed becomes the sieve tube proper; the smaller one, retaining its nucleus, becomes the companion cell. The number of companion cells formed by a single sieve-tube mother cell varies, but as a rule it is not more than one. Sometimes the mother cells do not undergo division, and thus there may occur a series of sieve tubes without companion cells. Besides sieve tubes and companion cells, we find conducting parenchyma in the phloem. These cells are not always distinguishable from the tubes in cross section, since they have about the same size and the same delicate walls. However, in radial section, they are seen to be elongated, rectangular cells, with end walls bearing simple pits very unlike the multiperforate end walls of the sieve tubes (Pl. 32, B).

The phloem fibers are long and awl-shaped, with much thickened secondary walls which later become lignified. A small lumen is usually present in these cells, but pits are wanting. The diameter of the cells varies greatly, fluctuating within the limits of 19 and 40 μ (Pl. 46, A, B).

The cells of the cambium (Pl. 32, D) are of the general shape and proportions of tracheids; the ends are pointed, the terminal walls following an oblique tangential course. In radial section the sloping character of the end walls is not apparent. In cross section (Pl. 30, A) the cambium cells are rectangular, with the greater diameter in the tangential direction. Secondary medullary rays, of course, arise in the cambium from typical cambium cells which undergo a definite number of transverse divisions. These medullary initials persist in the cambium and are seen in tangential section to constitute a part of that tissue (Pl. 32, D).

The remaining cells of the vascular cylinder, the endodermis and the pericycle, resemble closely the cells of these tissues as usually found in herbaceous dicotyledons. The elements of the pericycle are cylindrical parenchymatous cells which vary greatly in size. The endodermis as shown in Plate 29, D, is composed of a single layer of cells which differ

from the adjacent cortical cells in their smaller size, more regular arrangement, and lack of intercellular spaces. The tangential walls usually exceed in length the radial ones, though sometimes the cells are isodiametric. Casparian strips are present, but the lignified area is not always distinctly noticeable. In fixed material the protoplasm is found adhering to these strips, but withdrawn from the tangential walls, and thus forms two slime strings. The cells of the endodermis contain some starch even when all other tissue is empty (Pl. 31, A). This starch content is in some plants the most constant criterion for the identification of the endodermis, since the Casparian strips are not always distinguishable.

Both cortex and pith are made up of irregularly spherical, rather large cells interspersed with small, intercellular spaces. The pith often becomes hollow very early, but in certain varieties it remains almost intact until the plant is mature.

The cells of the collenchyma of the potato plant are prosenchymatous in nature, and the walls are thickened in a highly characteristic manner. The deposition of thickening layers is restricted to the corners of the cell and to certain places in the radial walls (Pl. 29, A, D), giving the lumen of the cell a more or less rounded outline in cross section.

Both the epidermis and the subepidermal layer are made up of brick-shaped cells. Those of the epidermis are more regular and often isodiametric; the tangential walls are slightly arched, the outer ones more than the inner. The outer wall has also a slightly developed cuticle. Here and there in epidermal cells anticlinal walls appear, suggesting late division among these cells. Some of the epidermal cells are specialized to form the guard cells of stomata. Beneath the stomata, chambers occur in the subepidermal cells (Pl. 32, A).

THE LEAF

The leaf, with its petiole, may be considered as a lateral expansion of the stem, and its tissue as derived from and continuous with the latter. As seen in cross section (Pl. 36, C), the vascular tissue of the petiole forms a semicircle, open toward the upper surface. The vascular bundles are surrounded on all sides by cortical tissue which merges into collenchyma just beneath the epidermis. Near the base of the leaf blade the outline of the cross section is semicircular; but toward the base the petiole gradually widens (Pl. 43, C). This widening and consequent flattening causes the amount of cortical tissue to decrease on the convex side—that is, the lower side—and on the flatter side gradually to increase. The vascular tissue consists of distinct groups (Pl. 43, C). The central group is relatively small; the two lateral groups, which are separated from the central one by more or less narrow gaps, are large. Isolated from these groups on each side there occur one or two small strands, which form the outer limit of the semicircle and lie in the petiolar wings. As regards the

detailed arrangement of these tissues, the condition found in the stem prevails (Pl. 36, A). The protoxylem is endarch, its first elements consisting of loosely ringed and spiral cells which are gradually superseded by larger closely ringed or spiral ones. Toward the outside, reticulate and porous vessels are found. Between the vessels, which are arranged in radial series, are uni- and bi-seriate medullary rays and small tracheids. The inner and outer phloem groups are in form and structure similar to those of the stem and will not be treated further. Throughout the petiole a cambium is developed which gives rise to some secondary growth (Pl. 36, A). The elements formed by the cambium are mostly vessels and tracheids, six or eight rows representing the extent of development by this meristem (Pl. 43, C).

The midrib projects both above and below from the surface of the lamina. On the lower side it is prominent and convex in outline. On the upper side it forms an indistinct flat ridge which is only noticeable in cross section of the leaf. As seen in cross section, the vascular tissue of the midrib, like that of the petiole, forms a semicircle, the open side toward the upper surface of the leaf. But here the vascular tissue is not at all, or only partially, broken into individual strands. The cortical tissue is differentiated near the epidermis to form a layer of collenchyma. This layer of collenchyma is rarely more than two cells thick except in the upper projecting ridge, which is composed almost wholly of this type of tissue. A cambium is rarely developed. The cells between the xylem and the outer phloem are parenchymatous and sometimes of rather uniform arrangement, closely resembling those of the cambium. Old material shows that these cells may give rise to some weak secondary growth, appearing most prominent at the base of the leaf, gradually disappearing along the rachis, and not extending to the terminal leaflet.

Both in petiole and midrib large ovoid parenchymatous cells are found between the external phloem groups. These cells are always present, though in varying amounts; their significance could not be determined.

The lateral veins are similar to the midrib in anatomy and morphological structure. The projecting ridges become reduced, and the amount of collenchyma is limited to one layer on the lower surface. The vascular tissue also decreases gradually with the size of the vein. The phloem groups become rarer, and the cells of each fewer. The xylem also becomes reduced till finally the terminal branchlets consist of one or two spiral elements and conducting parenchyma.

The mesophyll of the lamina consists of a palisade layer and spongy parenchyma (Pl. 36, E). The palisade tissue, which lies on the upper side, consists of a single layer of elongated and closely packed cells in uninterrupted contact with one another, except in mature leaves, where they are sometimes separated by narrow intercellular spaces. The palisade layer abuts upon the spongy parenchyma, the cells of which are irregular, loosely arranged, and poorly provided with chlorophyll.

Except for stomatal openings, the epidermis completely covers the leaf and is closely similar to that of the young stem. Stomata are found on both the lower and upper side, but are far more numerous on the lower surface. The stomata as seen in Plate 36, E, are of a simple type. The pores are surrounded by a pair of specialized guard cells which contain numerous chloroplasts; accessory cells are not present. The air chambers are small and are formed by the reduction in the size and by the arrangement of the subepidermal cells.

THE ROOT

A cross section through a small fibrous root (Pl. 37, B) shows a central core of vascular tissue, limited on the outside by an endodermis and cortex. The latter varies in extent with the size and age of the organ, being most prominent in young, small roots, and becoming less conspicuous in old, mature structures. The peripheral cells of the young cortex are covered by a "root epidermis," which, however, in old roots becomes torn and is sloughed off. The traces of vascular tissue supplying the lateral rootlets arise in the pericycle, and are given off directly and without branching or complication. Since the amount of primary structures is very insignificant, and most of the tissue of older roots is secondary in origin, the structure of the root will best be studied in its development, and for that reason will be discussed in detail in the section on ontogeny.

THE STOLONS

The stolons arise exogenously from the underground portion of the stem, which they resemble in structure and arrangement of tissues, except for a reduction in the amount of xylem, and absence of specialized mechanical tissue (Pl. 38, A). There are few xylem elements and these are vessels; tracheids are even less plentiful. Collenchyma is entirely wanting, and the epidermis does not show the specialization found in the aerial portion of the plant. The vascular strands of the stele, which when young are separated by gaps, are later incompletely united by means of an interfascicular cambium, which gives rise to some secondary growth. Fibers are found both in the inner and in the outer phloem, but they appear very late in the ontogeny of the organ. According to Reed (*11*), the endodermis here contains no starch even when the surrounding cortical tissue is crowded with starch grains, and this study confirms his observation, inasmuch as there is far less starch in the endodermis than elsewhere. The reverse is true for the endodermis of the stem. The cells of cortex and pith show no feature of interest, being of the type observed in the stem.

THE TUBER

The potato tuber is morphologically a shortened, thickened stem with scalelike leaves, or leaf scars. The eye in its entirety is a leaf scar with its subtended axil, which contains a suppressed lateral branch with

several axillary buds and undeveloped internodes. The central bud of an eye is most prominent and develops first upon renewal of growth. The spiral of the eyes of the tuber is usually left, though De Vries (7) records right spirals also, the latter type less frequent.

Sections through the mature tuber show several zones of tissue readily distinguishable to the naked eye. These zones are the cortex with its periderm, the vascular ring, and the pith. Of these three areas the vascular tissue is least, the pith most prominent. In the region of the eye the vascular tissue approaches the surface of the tuber and provides vascular connection between the developing buds and the reserve materials stored in the tuber.

The amount of the vascular tissue of the tuber is only slightly greater than that of the stolon; but the individual groups are much separated in the expanded tuber, being only here and there united by interfascicular cambium. The xylem is mostly primary in nature, and only in the region of the larger groups are porous vessels of secondary xylem found. As will be shown in the developmental study of the tuber, the phloem becomes more and more broken up into small strands which are found scattered throughout the cortex and pith. The cortex and the pith differ mainly in the relative amount of cellular density, the cortex being more dense on account of the smaller size of its cells and the larger amount of cell content other than starch.

A periderm 6 to 10 cells in thickness covers the entire tuber. The homogeneity of this layer is broken by small lenticel-like structures which are concerned with the aeration and which have developed below the position of the stomata of the young stolon tip.

THE FLOWER

The flowers are borne on short bractless pedicels which show the histological features characteristic of the stem (Pl. 40, A). The vascular tissue, however, forms a more or less continuous band instead of being arranged in distinct groups (fig. 2, A, a). With the broadening of the pedicel to form the torus of the flower the band of vascular tissue becomes broken through the separation of five vascular strands (fig. 2, B, b), which diverge to occupy a position in the outer cortex—that is, the peripheral region of the cortex (fig. 2, C, b).

When these groups have become distinct—in fact, even a little earlier—almost all of the remaining vascular tissue, c, of the cylinder now more or less reunited (fig. 2, D, c), passes out obliquely to form 10 separate bundles, d, in the inner cortex (fig. 2, E, d). The tissue which does not pass out is in two elongated groups which soon divide (fig. 2, E, e), each giving rise to two small groups of unequal size which, when entirely free, occupy the four quadrants of a circle (fig. 2, E, e; F, e).

Of the 10 inner cortical groups, d, the five which alternate with b begin to expand and divide (fig. 2, F, f). Each one cuts off by radial

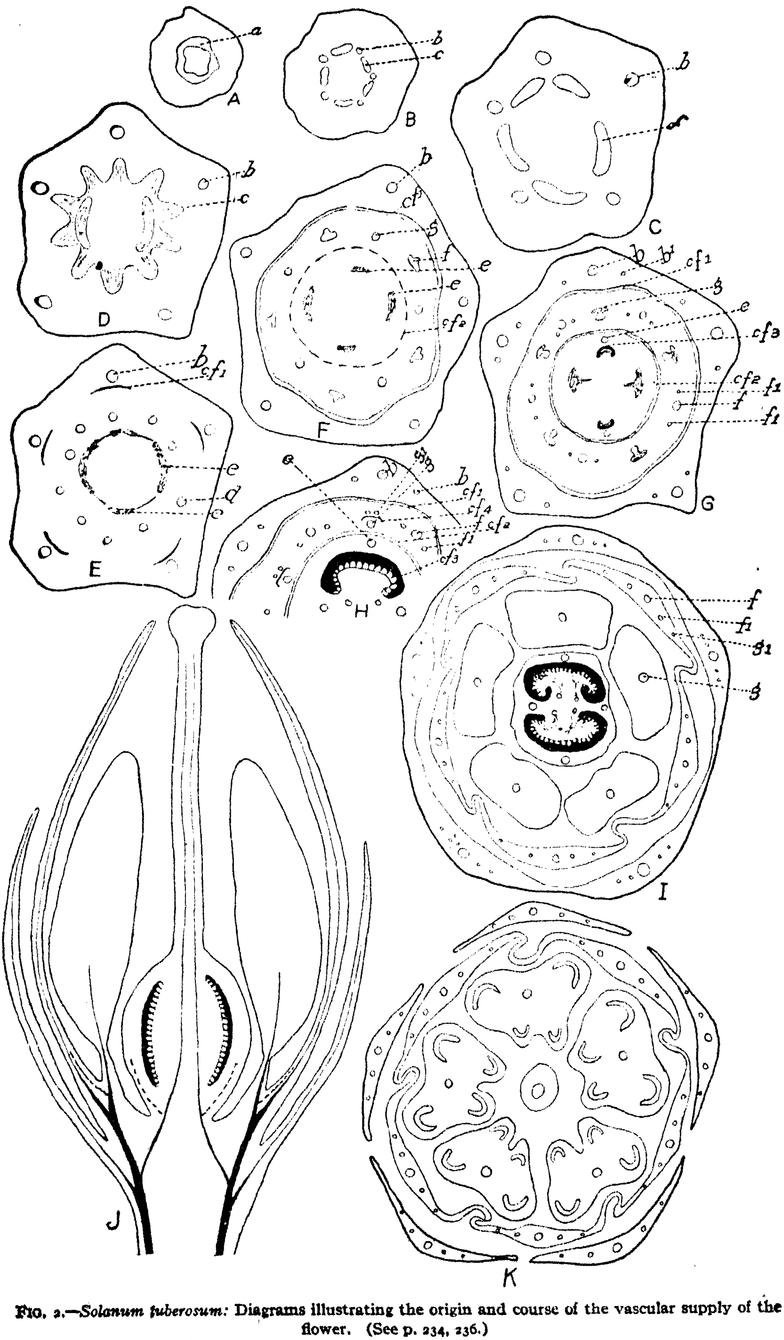

FIG. 2.—*Solanum tuberosum:* Diagrams illustrating the origin and course of the vascular supply of the flower. (See p. 234, 236.)

constriction two small ones, f^1, on opposite sides of a large third one, f, the remainder of the original group (fig. 2. G).

Simultaneously with these divisions a cleavage furrow, cf^1, appears inside each of the five outer cortical groups, b, as shown in figure 2, E. These furrows extend laterally until they meet, cutting off a concentric ring of tissue which represents the first cycle of the flower—that is, the calyx (fig. 2, F).

When the five inner cortical groups, f (fig. 2, G), have completed division, a second set of cleavage furrows, cf^2, originates, cutting off another concentric ring of tissue and leaving in the center a slightly oval area, in which are found the four vascular groups, e.

Simultaneous and progressive changes are now seen in all parts. In the calyx the midrib, b, has given rise to groups of vascular tissue (fig. 2, G, b^1), which in some places are still connected, in other places are already distinct, forming the lateral veins of the calyx lobes. Each of the five bundles (fig. 2, F, g) of the inner cortex which lie opposite the midribs of the calyx lobes cuts off a small amount of vascular tissue to form two distinct bundles (fig. 2, H, g^1). The central portion of the tissue of the torus, which is not differentiated, and which contains the innermost ring of the four bundles, e, now begins to form the ovary proper (fig. 2, G). The first visible change is the appearance of two small openings (fig. 2, G, cf^3), the convex sides of which are directed toward the two smaller of the four vascular groups, e, just mentioned. Serial sections show that these openings become larger; the surrounding tissue becomes the two carpels of the ovary which are united adaxially. The margins of the coherent carpels form more or less evident outgrowths, the placentas, which in turn bear the ovules.

As soon as the cortical groups (fig. 2, H, g^1) have become distinct, a cleavage furrow (fig. 2, H, cf^4) begins to form just below them and gradually advancing tangentially cuts off the second cycle of the flower—that is, the corolla. This cycle is not of equal width throughout, but is constricted and folded at five places to allow for the later expansion of the wheel-shaped corolla (fig. 2, I). The midribs and larger lateral veins of these lobes of the corolla tube are formed by the inner cortical groups f and f^1. The vascular tissue of the smaller veins of the corolla is derived from the five inner cortical groups, g, which were last to divide.

The tissue between ovary and corolla becomes radially cleft to form the five stamens. Each stamen has for its vascular supply a single strand of tissue which is derived from one of the five inner cortical groups, g, opposite the calyx lobes (fig. 2, I, g).

The course of the vascular supply in the flower and the mode of origin and course of the traces supplying the different members of the same is illustrated in the semidiagrammatic sketch (fig. 2, J). Figure K shows a mature though still unopened flower, with its vascular supply in cross section through the anthers just above the distal end of the ovary.

ONTOGENY

THE STEM

A section taken from the tip of a growing potato sprout shows that this region displays no trace of the complicated structure of the older portion of the stem, but is entirely made up of thin-walled cells which are rich in content. Close behind the meristem or true growing region, the uniform cell mass becomes differentiated into distinct layers. The cells, however, retain the abundance of protoplasm, the thinness of the wall, and also the power of division. Farther away from the growing point, the distinctive characters of the tissues become more and more apparent, the organ gradually attaining the differentiation of the mature plant.

The first differentiation in the distal end of a developing sprout consists in the setting off of three distinct regions: the dermatogen, the procambium, and fundamental tissue, including cortex and pith (Pl. 41, A, D). The procambium forms an unbroken hollow cylinder, with small projections into the pith. It is made up of small and elongated thin-walled cells, with abundant protoplasmic content, thereby differing from the surrounding cells of cortex and pith, which are much larger and short cylindrical in vertical section. Almost simultaneous with the setting off of these tissue regions, the first elements of mature vascular tissue appear. They are found most commonly in the small inner projections of the procambium cylinder (Pl. 42, A, D), and are recognized by their slightly larger size and by the secondary thickening of the wall. Longitudinal sections show that these cells are longer than those of the procambium, and further, that the secondary thickenings consist of simple rings located rather distantly from one another. The youngest material examined showed six to eight such elements in one cross section (fig. 3, A). These cells, the first of the protoxylem, then, are the first vascular elements to differentiate from the procambium. It is, however, generally held that the phloem is differentiated at an even earlier period than the xylem, but if such is the case, the phloem cells are not distinguishable from the surrounding procambium cells.

A period during which both growth and differentiation reach their greatest intensity now ensues. The changes consist chiefly of progressive growth and maturation in the procambium cylinder. The latter increases in actual size, both by cell division and cell enlargement. This increase in size of certain of the elements is most marked in the procambium cells at the periphery of the pith, resulting in the setting off of small groups of cells which have not enlarged; the latter cells are the internal phloem group initials (fig. 3, B; Pl. 41, C; 42, B, D). A number of cells near the middle of the procambium cylinder stand out clearly because of their more regular size and orderly arrangement in the form of a tangential band one cell wide. This band, however, is not continuous, but is evident only in those places opposite the procambium

projections (fig. 3, D). More advanced stages in the differentiation of the procambium show these calls to be cambium initials.

While these changes are taking place the number of xylem elements is increased. The later-formed cells appear progressively farther and farther away from the pith, thereby indicating that the development of the protoxylem is proceeding centrifugally (fig. 3, C). Aside from a difference in position, the later-formed protoxylem is characterized by larger size and by a different type of secondary thickening in the form of loose spirals and close rings.

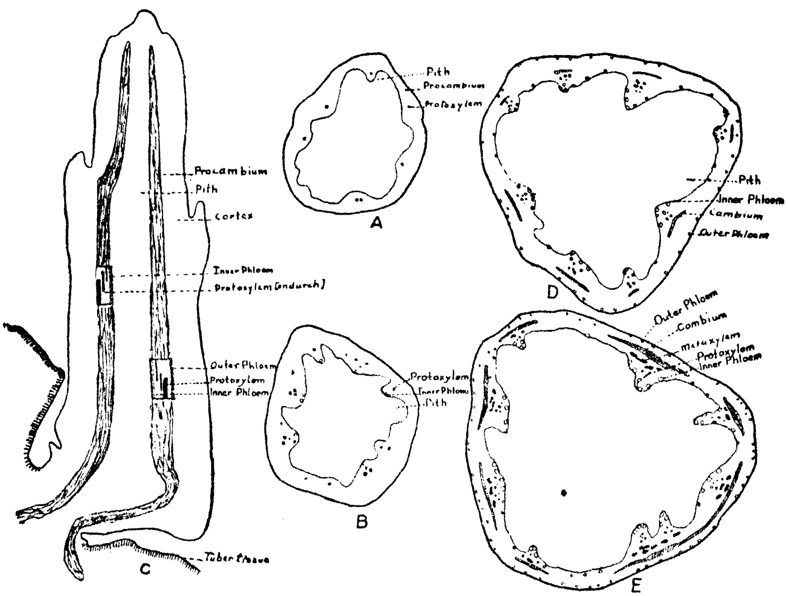

FIG. 3.—*Solanum tuberosum:* Diagram illustrating the mode or origin, orientation, and development of the vascular tissue of the stem. A, B, transverse sections through the distal end of a potato sprout; C, radial section through such a sprout showing vascular connection with mother tuber; D, E, transverse sections farther away from the growing point.

Growth and differentiation become more and more evident; these consist of further changes in the regions already set off, and of specialization in the still undifferentiated part of the procambium. In the external region distinct differentiation also now occurs. The changes, though similar, are less extensive than those noted at an earlier period in the procambial region adjacent to the pith. Thus, a small number of phloem group initials is set off; the number of cells in each of these is also small (fig. 3, D; Pl. 42, B).

Three types of primary vascular tissue are thus early differentiated from the procambium: protoxylem, cambium, and phloem. The phloem groups appear in the innermost and outermost regions of the procambium; the bundles so formed are therefore bicollateral (Pl. 41, B).

Though the protoxylem elements are easily recognized in cross section by the thickness of their secondary walls, the protophloem cells have little to distinguish them from the procambium except their location. In vertical section, however, they do exhibit a few differences, though only under high power. The cells of the inner phloem which lie adjacent to the pith, though having the general proportions of the normal procambium cell, are slightly more elongated, and the content of the cells is richer and more granular. In fixed material the protoplasm has withdrawn from the side walls and has formed a slime string, which in the region of the transverse wall of the cell widens, filling almost the entire lumen. A similar condition is found in the adjacent cell, suggesting a protoplasmic connection between these cells, but actual perforations in the wall are not noticeable as yet. These elements are the first sieve tubes of the phloem, and from their location, it is noticed that they have developed farthest away from the protoxylem elements, differentiating progressively outward. Their development is, then, centrifugal, like that of the protoxylem. A little later, sieve tubes in the outer phloem begin to appear. These are similar in structure in every way to those of the inner phloem, but develop centripetally (fig. 3, D). The sieve tubes of the inner phloem are, then, the first to differentiate, a fact which might have been expected, since here the first visible differentiation of phloem group initials takes place. The number of sieve tubes at such a stage is very small, for rarely are more than one or two parallel rows seen in a section.

A cambium becomes distinct very early in the ontogeny of the stem even before the sieve tubes of the inner phloem are differentiated. The first-formed cambium initials divide rapidly forming two or three tiers of cells in the region of the projecting points of the procambium, long before a tangential connection between all the cells of this layer is established.

The protoxylem elements, maturing at this stage, are still small; they have secondary thickenings in the form of close rings and flat spirals. The secondary thickenings of the first-formed elements, owing to constant elongation of the cells, have become separated and form scattered rings (Pl. 32, D). They are further distinguished from the later-formed elements by their location and smaller size.

In somewhat older stages several changes in progressive differentiation have taken place. Plate 41, B, shows the vascular cylinder at such a stage. The number of protoxylem elements has increased from about 8 to 56, and the phloem groups have more than doubled in number. Though it is not always possible to distinguish the individual groups in their entirety, 76 groups were counted in the inner and 68 in the outer region (fig. 3, E). Still farther away from the distal end is found what is probably the maximum number of phloem groups, 116 in the inner

and 102 in the outer region. The number of protoxylem elements at this stage is about 130.

On taking the whole of a cross-sectional area at this stage, it is noticeable that there has been an unequal differentiation in the procambium cylinder, with greater development and specialization in the region of the procambial projections. These regions have developed to an extent such that under low power six groups of vascular tissue and gaps separating them may be readily differentiated. Gradually, however, differentiation extends also to these interfascicular regions, being initiated by the appearance of a cambium and primary phloem group initials. Plate 31, A, shows the development of such an interfascicular cambium. It does not form simultaneously throughout, but arises in different places, gradually uniting and thereby bridging the gaps between the large groups of vascular strands. Phloem groups are seen outside and inside the cambium. The outer groups are small and closely arranged (Pl. 29, A, C), the inner more or less scattered and usually more distant from the cambium, forming, together with those of the inside of the projections, a more or less symmetrical figure.

In the region of the larger vascular groups large xylem initials are formed (Pl. 29, C; 42, A). The thin delicate primary wall of these cells soon becomes strengthened by secondary thickenings in the form of scalariform and reticulate bands. In vertical section these cells are cylindrical with somewhat sloping end walls. The type of secondary thickening, unlike that found in the protoxylem elements, is such that further elongation of the cell is impossible. This fact, together with difference in size and location, serves to distinguish between protoxylem and these later-formed, larger cells of the primary xylem which are known as metaxylem.

The nature and amount of differentiation which we have thus far followed in the young potato sprout relate to the procambium, which, as we have seen, gives rise to all the primary vascular tissue of the stele. Simultaneous changes in the other meristematic tissues consist chiefly of cell enlargement without marked differentiation. In the cortical region the peripheral tissue undergoes qualitative but not quantitative changes. The two outermost layers are of cells, rather regularly arranged, the elements themselves being more or less rectangular and vertically elongated. Within these we have two or three layers of cells, polyhedral in cross section, and tracheid-like in tangential view. The walls of these cells, however, are thin and of cellulose. With the appearance of the potato sprout above the surface of the soil, these prosenchymatous cells of the cortex develop secondary thickenings in a characteristic manner. Wall thickenings occur in the four corners, sometimes also along the radial walls (Pl. 29, A, D). This specialized tissue of the cortex, the collenchyma, serves as the supporting tissue of delicate, growing organs.

Differentiation of the vascular cylinder continues. The first formed metaxylem elements are now mature; new ones appear continuously.

These are arranged in no constant or typical manner, though often the larger cells form radial rows (Pl. 42, A).

The inner phloem groups meanwhile increase in size and in number of elements. Sieve plates are readily seen in both longitudinal and cross section. The outer phloem groups also become larger and more distinct. In many places they are separated by large, ovoid cells. The interfascicular cambium is almost completed between the six first-matured groups of vascular tissue, and is in places two or three rows wide (Pl. 29, D).

An endodermis has so far not been distinguishable from the cortex, but now becomes fairly distinct. The cells making up this layer are small, usually more regular in shape, and contain starch even when the latter is not present in other tissues. (Pl. 29, C; 31, A.)

The phloem fibers are the last to appear. The first of these are differentiated simultaneously in the inner and outer phloem. At first they are seen singly; later they may increase in number forming groups. Their walls are usually heavily thickened, but do not become lignified until later. In the inner phloem the fibers usually appear in groups either scattered among the peripheral pith cells or forming the inner limit of the phloem groups (Pl. 33, C). In the outer region the fibers usually appear in groups, either scattered among the peripheral pith cells or abutting on the inner phloem groups (Pl. 33, A). In the outer region the fibers form a single broken layer next to the endodermis; they also occur occasionally in groups.

Not all of the procambium cylinder differentiates into conductive elements and fibers. Some of the cells of the outer region enlarge without specialization, forming the parenchyma between the outer phloem groups, the pericyclic region of the stele. In the inner region a similar change takes place. The cells between and around the protoxylem elements and the innermost phloem groups, though differing from the pith cells in being smaller, remain parenchymatous and unspecialized. They form, together with the protoxylem, a band of tissue limiting the pith on the outside and the vascular cylinder on the inside, a band which may be called the "perimedullary zone," or "*Markkrone*" (Pl. 29, C; 30, B). Sometimes typical pith cells separate groups of inner phloem from this region. These isolated phloem groups then appear as though they do not belong to the stele (Pl. 29, A), and, hence, have been called "pith bundles."

Above is in brief the early ontogeny of the tissues and elements of the sprout of the potato plant. In comparison it is of interest to trace the development of a growing tip of a mature stem, and to note differences in order of appearance.

In such an older stem there is already very near the growing point a fairly well differentiated vascular ring. The inner phloem groups are distinct and numerous; the outer groups are still undifferentiated procambium. The protoxylem elements are found singly and scattered;

they are small and few in number. Here differentiation of the phloem seems to be in advance of that of the xylem, or at least it has kept pace with the latter, whereas in the growing tip of a sprout no phloem is found when the first protoxylem elements have become evident. Even at these early stages collenchyma is present. This tissue is obviously here as a supporting structure, since in the underground sprout such tissue is not found. An endodermis has also become visible about this time. The cells are recognized by their regularity, smaller size, and starch content; Casparian strips are not developed.

The other tissues develop in the same sequence as that described in detail for the sprout.

THE LEAF

The petiole and leaf blade may be considered as morphologically a lateral expansion of the stem, and their tissues as continuous with and derived from the latter. A good discussion of the gross morphology of these organs is found in De Vries (*8*). The substance of this is given here, and is followed by the writer's observation on the structure and development of the internal parts.

The leaf primordia appear on the vegetative cone as small protuberances which soon push farther out and curve slightly, the adaxial surface becoming slightly concave. Continuing in this increase and curve, the primordia soon bend over the growing tip, their form thus constantly changing. This cone-shaped growing point, made up of the primordia, bulges on two sides, and soon these swellings can be recognized as the future blades of terminal leaflets. In a very early stage the terminal leaflet consists of a short petiole, a heavy midrib, and blade halves folded together adaxially. For a long time the terminal leaflet is far in advance of the other organs in its development. The latter differentiate only gradually, keeping pace with the elongation of the stem, and thereby providing space for the primordia of the lateral leaflets. The difference in rate of development is so great that the terminal leaflet is already 1 cm. in length and has become green long before the rest of the leaflets appear. The development of the lateral leaflets takes place in basipetal succession; consequently the uppermost leaflets are already green and well grown when the lowermost are still primordia. These lateral leaflets appear as protuberances on the petiole in the same manner as the primordium of the leaf itself appears on the vegetative cone. These are at first quite small, but soon elongate and pass through the same stages as do the halves of the terminal leaf blades.

The first leaf hairs appear very early, developing acropetally on the convex surface of the primordium; later they are formed also on the inner surface in the same order. The hairs appear in two longitudinal rows on the veins, but none are found on the lamina itself. At first only glandular hairs are formed, but soon simple stiff hairs also develop. Both types increase rapidly in number and are mature long before the

internal differentiation of the leaf is complete. When the leaf is still small, the hairs are very numerous and the epidermis over the veins is covered with a dense mat of them; with the elongation of the individual organs the thickness of this mat decreases, since between the already existing hairs no new ones are formed. On the mature leaf the hairs are widely scattered.

Tissue differentiation in petiole, midrib, and veins follows the same general order as described for the stem, but the location and the relative amount and size of the elements is somewhat different. Sections through the middle of a leaf primordium show a small crescent-shaped mass of procambial tissue surrounded by large cells of the fundamental meristem. At first only slight differentiation is noticeable; in about the middle of the procambium a very few protoxylem elements appear. A little farther back from the growing point specialization in the peripheral procambium is going on; the cells are increasing in number and their nuclei are large. Those near the protoxylem at the same time expand and become arranged more or less in tangential rows.

Near the base of a leaf primordium a well-developed procambium area with about eight protoxylem elements is found. At the periphery of the procambium, which has now become semicircular in section, phloem initials appear, forming a band of smaller cells. The first differentiation of the phloem into groups is noticeable in the internal phloem (on the upper side of the procambium). These groups are few and large, and make up a large proportion of the vascular tissue. Here and there the outer phloem (that toward the lower side of the leaf) which had appeared from the procambium as a nearly continuous band, is separated into very small groups. There is, however, considerable variation in the condition found in different leaves of the same size. Often the procambium, though specialized at the periphery, has not as yet formed phloem group initials, whereas in other leaves of the same size almost complete differentiation of the phloem groups has occurred.

In sections through older leaf primordia an increase in the number of protoxylem elements occurs, and the first metaxylem initials, which can be readily distinguished by their large size, have also begun to appear. The cells of that portion of the procambium which has not yet become specialized to form either phloem or xylem, increase in number and size. Those which lie along the convex side of the vascular semicircle have a somewhat regular arrangement thus foreshadowing the appearance of a cambium. Between the outer phloem initials large oval cells become noticeable (Pl. 36, A). Their appearance and location is so characteristic and constant that they would seem to deserve greater attention. Serial sections, however, show nothing suggesting function or structure different from that of the cortical cells. Sections through more advanced stages show both outer and inner phloem arranged in groups which are now quite distinct, and only in the region of the ends

of the crescent do these groups unite, thereby establishing a vascular connection between inner and outer phloem (Pl. 36, A, C). A cambium is developed throughout the crescent of vascular tissue, being more prominent in the region where the procambium has given rise to the largest amount of vascular tissue and less in those regions where specialization is very slight. This cambium later gives rise to some secondary growth (Pl. 44, D).

With the appearance of the first distinct phloem groups, differentiation in the leaf blade begins. Up to this time the tissue of the leaf blade is homogeneous in structure, being made up of brick-shaped cells (Pl. 36, B). An epidermis soon becomes distinguishable and stomata develop. The first cells of the blade to change are those just beneath the upper epidermis. These cells elongate to twice their former length and remain closely packed, forming the palisade initials (Pl. 36, D); in the lower tissue intercellular spaces begin to form, but the size and shape of the cells remain nearly the same. The leaf blade is fully developed, and the cells have completely matured before differentiation in the veins has ceased. The mature leaf blade (Pl. 36, E) has a well-developed epidermis, a palisade tissue one cell deep on the upper side, and three to five rows of spongy parenchyma the cells of which are separated by numerous and large intercellular spaces.

THE ROOT

In plants grown from tubers all of the roots are fibrous in nature, and arise endogenously from the nodal pericycle of the subterranean part of the stem (Pl. 37, A). Transverse sections through this region in a young sprout developing beneath the ground show cells of the pericycle opposite the protoxylem groups richer in content and undergoing division. The cells first elongate radially, and then divide tangentially. The meristematic masses so formed are the root primordia; the central cells of this tissue become the vascular tissue of the young rootlet. The endodermis just opposite the root increases in extent, pushing out as a lobe ahead of the developing rootlet. The latter pushes its way mechanically through the cortex, and is aided by the dissolving action of enzyms which are probably secreted by the cells of the endodermis. Just before the epidermis of the stem is broken the cells of the endodermis cease division and are ruptured, giving way to the rootlet, which then penetrates to the surface and develops independently in the soil.

Transverse sections near the tip of a rootlet which has just broken through the cortex show several distinct zones of tissue. The innermost region, which occupies only a small area, is a solid strand of primary vascular tissue separated from the thick cortex by an endodermis. The epidermis at this stage is specialized, in that many of its cells are elongated to form the root hairs.

The vascular tissue is arranged radially, as is usual in roots, the xylem and phloem in separate strands. These strands alternate with one

another, and the xylem abuts directly on the endodermis. The number of these varies; lateral roots are usually, perhaps always, diarch (Pl. 37, C); others may possess more than these. The maturing of the vascular elements, however, has proceeded in a different direction than was observed in the stem. The first protoxylem elements to mature are those farthest away from the center; the development then is centripetal. The later-formed protoxylem elements of the two groups approach each other more and more closely with the increasing age of the region; those last formed meet in the center. Sometimes, however, a few cells in the center do not become vascular tissue, but remain parenchymatous, forming a pith.

The protoxylem forms two small groups of tissue, somewhat oval in shape, which are separated from the diarch xylem mass by a parenchymatous sheath. Very early a cambium appears; this forms a complete cylinder lying outside the xylem and inside the phloem. Cells are produced by this cambium more rapidly in the latter region and the cylinder soon becomes symmetrical. This secondary tissue is collateral, whereas the primary is radial.

With the continuance of secondary growth, the amount of vascular tissue relative to that of the cortex increases rapidly. Instead of the small vascular cylinder and the huge cortex of the very young root (Pl. 37, C), in the old root there is a large amount of vascular tissue with a comparatively narrow cortex (Pl. 37, D). Most of the tissue of the root is, then, secondary in origin.

The secondary wood of the root consists largely of vessels and wood parenchyma. The vessels are porous and either very large or very small. The large ones are arranged more or less in radial rows; the spaces between them are chiefly filled by the smaller type of vessel. The wood parenchyma, although somewhat scattered, is usually found around the large vessels—that is, it is vasicentric. Occasionally the lumen of one of the large vessels is blocked by bladder-shaped intrusions derived from the membranes of the pits between the vessels and the adjoining parenchyma cells. These vesicles, which are known as tyloses, are very common in many plants, but in the normal potato plant they are not often observed except in the root. The medullary rays of the mature root are few and uniseriate (Pl. 37, D). Typical tracheids are absent, but a few thin-walled fibers are found scattered between the vessels.

Just as most of the xylem is secondary in origin, so is the phloem made up almost entirely of secondary elements. The primary phloem is not recognizable in the mature organ; its cells have become nonfunctional and later are crushed. Most of the phloem cells formed by the cambium are sieve tubes (Pl. 44, C), with their respective companion cells. The sieve tubes are larger than those found in the stem; in general proportion and arrangement, however, they do not deviate from the latter. The cells of the medullary rays of the phloem are slightly broader than those

found in the xylem; their number, of course, is very small. Phloem fibers, as might be expected, are not found in the root.

The cells of the cortex and endodermis are of the type described in detail for the stem. The outer cells of this tissue, since they are not protected by a specialized dermal layer, become somewhat torn and their walls suberized.

THE HYPOCOTYL

Since the change from the exarch condition of the root to the endarch condition of the stem takes place in the region of the hypocotyl, seedlings instead of sprouts grown from tubers had to furnish the material for investigation. The primary vascular tissue of the root develops centripetally, the latest protoxylem elements to mature being found near the center (fig. 4, a).

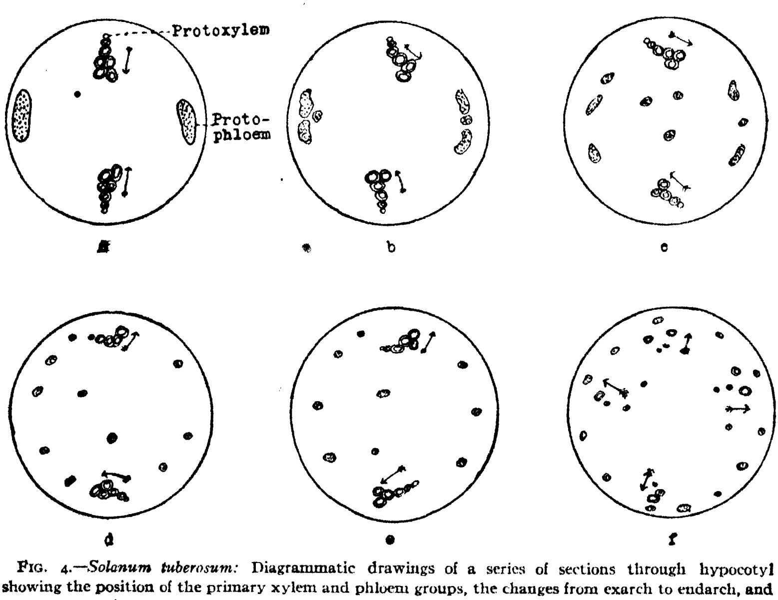

FIG. 4.—*Solanum tuberosum:* Diagrammatic drawings of a series of sections through hypocotyl showing the position of the primary xylem and phloem groups, the changes from exarch to endarch, and the behavior of the phloem.

In the stem the condition is reversed, for here the maturing of the elements takes place centrifugally, and consequently the smallest protoxylem elements are found near the center. In the change from the exarch to the endarch condition it is noticed first, that the two protoxylem groups of a diarch root begin to swing outward, one group following a left, the other a right curve (fig. 4, b). In the region just below the cotyledons the bending has progressed so far that the protoxylem groups instead of forming a radial row, come to lie in a tangential plane. In the region above the cotyledons the change from the exarch to the endarch condition is complete.

Simultaneously with the change in the orientation of the protoxylem in the hypocotyl, go different, though not less important, changes in the phloem. In the root the primary phloem alternates with the xylem, both tissues being arranged in distinct groups. In the stem the arrangement is bicollateral—phloem both inside and outside the xylem. The first noticeable change in the hypocotyl consists in the breaking up of the two phloem groups of the rootlet, with the formation of three to four smaller groups. These phloem strands orient themselves in such a way that two or three of the groups come to lie in the center of the stem between the now separating xylem groups (fig. 4, b, c). The other phloem groups take a position at the periphery of the stele close to the endodermis. At this stage the protoxylem groups have moved through an angle of 90°, and form a tangential row of cells. The outer phloem groups have meanwhile increased further in number so that some of the strands come to lie at the outer face of the protoxylem. The central groups of phloem have also divided and an increase in the size and number of the parenchyma cells in the center of the stele is forcing these groups away from the center and causing them to come to lie close to the protoxylem. The bicollateral condition is thus established (fig. 4, d, e, f).

STOLON AND TUBER

About six weeks after the potato tubers have been planted, branches arise in the axils of scaly leaves of the subterranean part of the stem and grow more or less horizontally outward. These branches, known as stolons, remain simple or fork and sooner or later swell at their tips to form tubers.

The stolons, being modified stems, present a typical stem structure and the tissues go through the same process of development as do those of the latter. In the study of the development of the tuber it is necessary only to note the changes incidental to the enlargement of the stem. In the mature stolon, then, is found a ring of vascular bundles consisting of four or five larger groups with a few smaller ones between them (Pl. 38, A). The xylem is only weakly developed and is made up chiefly of primary elements, among which are a few vessels. The phloem, however, is extensive, and the groups show the typical arrangement in both inner and outer region. The pith and the cortex are made up of large polyhedral cells, many of which are filled with crystal sand (calcium oxalate). A collenchyma and a specialized epidermis are not developed.

The first change in the tip of the stolon consists of extensive cell division in the region of the pith and to a less extent of the cortex, resulting in a swelling of the stolon, which becomes at first oval, later spherical, the change from stolon to tuber being quite abrupt. This excessive cell division in the region of the pith causes the vascular tissue to bend outward; transverse sections at the proximal end of the tuber show the vascular tissue cut obliquely, in places even longitudinally. Potato

tubers of this stage are quite small, not over 5 to 10 mm. thick. The tuber increases both in length and in thickness; the latter interests us chiefly. From this stage on the parenchyma cells of the perimedullary zone, and to some extent those of the cortex also, contribute mostly to the formation of the tuber tissue. The vascular cylinder is forced more and more outward by excessive cell divisions in the regions of the perimedullary zone and pith. However, all of the vascular tissue does not partake in this move. The inner phloem groups become separated with the increase in the tissue of the perimedullary zone and peripheral pith, and gradually split up into numerous strands which traverse these regions of active growth in all directions (Pl. 38, B, C, D).

An examination of the mature potato tuber leads to the conclusion that most of the tuber tissue is derived from the parenchyma of the perimedullary zone of the stele, to a less extent from the parenchyma of the external phloem, the cortex, and the pith. The amount of secondary elements added as a result of the activity of the cambium is insignificant and is limited to a few vessels and some wood parenchyma.

Reed (*11*), who first followed in detail the development of the potato tuber, maintains that the pith and the perimedullary zone contribute equally to the formation of new tuber tissue; but this does not seem to be the case, since even mature potatoes (in stained sections) show phloem strands in the region near the center—that is, all of the tissue of the tuber except that of the most central part and of the cortex is traversed by phloem strands. The view of De Vries and others that the tuber is formed largely by the activity of the cambium is no longer tenable.

The young tuber has a more or less distinct epidermis with scattered stomata (Pl. 39, A, B). Upon the enlargement of the tuber the epidermis undergoes marked changes. At first anticlinal walls appear in a few of the epidermal cells, probably as a sequence of the tension caused by the expansion of the organs (Pl. 39, C). Later periclinal walls also appear (Pl. 39, D). Simultaneously with the division in the cells of the epidermis division walls also appear in the subepidermal layer. Cell division in this region continues until a layer of tissue is produced which takes over the protective function of the epidermis; this is generally known as periderm or "cork" (Pl. 39, F). The formation and constant regeneration of the periderm are due to the activity of the meristematic cell layer known as the phellogen. In the potato tuber the phellogen consists of a single layer of thin-walled cells which divide tangentially and which constitute the inner row of daughter cells produced by the first division of the cells of the hypodermal layer (Pl. 39, D, E). While most of the periderm arises from the phellogen derived from the hypodermis, the epidermis gives rise to a superficial periderm usually three to four cells in extent. Both layers of periderm tissue, though adjacent to each other, are distinct. The periderm is perforated by a number of lenticel-like structures which arise immediately beneath the stomata, the function of which they assume.

SECONDARY GROWTH

As soon as the intercalary cambium has united the large vascular groups, sometimes even earlier, secondary growth becomes markedly evident. In its beginning the activity of the cambium is noticed only in the region of the large bundles of the stem and is extended only gradually to the interfascicular region, as described in the early part of this paper.

A fully mature stem shows a cylinder of vascular tissue which in the region of the large corner bundles is often more than 2 mm. thick (Pl. 43, B). The xylem in this region contains many large, porous vessels which are more or less regularly arranged. The spaces between the vessels are occupied by tracheids and wood parenchyma. The latter tissue, however, is small in extent, the cells being most commonly found around the large vessels (Pl. 44, A), as is the case in the roots. Medullary rays are very numerous, but the individual ray is narrow, being rarely more than one or two cells wide.

The interfascicular xylem differs from that found in the corners, in that it contains no large vessels, but is made up chiefly of a uniformly arranged mass of tracheids traversed by uniseriate medullary rays (Pl. 44, B). The first-formed xylem elements of this region have smaller lumina and much thicker walls than those later formed. A section through this region resembles strikingly a section of the xylem of a woody stem showing spring and summer growth reversed (Pl. 44, B).

The cambium gives rise on the outside to a comparatively broad ring of phloem which consists mainly of sieve tubes and medullary rays (Pl. 45, A; 47, A). The amount of this secondary phloem varies with the individual and with the place where the section is taken. In the region of the node (Pl. 43, A) the amount usually exceeds that found in the internode (Pl. 43, B); and in a given section the largest amount is found on the face of the large corner bundles. The medullary rays of the phloem widen as they approach the endodermis, and they often bend toward each other in pairs at their tips, inclosing a triangular area of tissue which is made up almost entirely of secondary sieve tubes (Pl. 33, A; 45, B). The primary phloem groups remain functional even after the formation of secondary phloem, and continue active up to the time of maturity of the plant. Their delicate walls, of course, become slightly thickened and occasionally calluses close a sieve plate; the latter, however, occurs only rarely and is probably a pathological condition (Pl. 46, A, B; 47, B).

The cambium gradually diminishes in extent. By the time the vines die, the cambium is in places entirely disposed of—that is, the cells have matured as either phloem or xylem cells.

With an increase in the amount of vascular tissue, the cortex and the pith undergo structural changes. In the region of the large bundles the cells of the cortex parenchyma have become flattened radially owing to

the tangential stretching resulting from an expansion of the vascular tissue which is not accompanied by a corresponding enlargement of the cortex itself. Whenever the expansion is too great to be compensated for by passive stretching of the cells, some of them become meristematic and divide by the formation of anticlinal walls. In the interfascicular region the expansion is much less, and consequently the cells of the cortex retain more or less their original shape. The boundaries of both regions may show transition stages, depending on the amount of secondary growth in the interfascicular region.

The secondary growth which is found in organs other than the stem has received consideration in the chapters on the ontogeny and will not be treated further.

GENERAL DISCUSSION

In the study of the anatomy and the ontogeny of the potato plant as presented above there are given a number of features sufficiently striking to justify reconsideration and discussion.

The protoxylem matures before the protophloem; it is perhaps even first to differentiate. It is usually stated that the phloem precedes the xylem in appearance in the higher vascular plants. The phloem initials appear at first in the inner, then in the outer region, and not in the reverse order, as stated by Weiss (*10*), for the Solanaceae. The maturing of the elements follows the same sequence. The primary phloem groups do not arise by the division of single initial cells, but from groups of small cells set off by the enlargement of surrounding procambial cells. Once set off, these groups enlarge by the formation of new elements.

The bicollateral condition, of course, is characteristic of the Solanaceae, but the inner phloem groups are usually limited to the peripheral region of the pith; sometimes, however, they are found farther away and near the center of the stem, a condition often observed in the potato plant. These innermost phloem groups clearly belong to the stele proper, and do not represent the vestigial remains of a second set of vascular bundles, as is thought by Worsdell (*12*) to be the case in the cucurbits. Their position near the center of the pith is accounted for as follows: A number of the parenchymatous cells of the perimedullary zone divide repeatedly in the radial direction, causing some of the innermost groups to be deflected from their straight course and take a position far within the pith. This type of tissue increase is characteristic of the thickening of the tuber and results in this case in the formation of the extensive sheath of parenchymatous cells which is traversed by numerous small phloem strands.

Of special interest also is the question of the extent of union of the individual phloem groups as they traverse the stem. An examination of Plates 34, A and B, and 35, A and B, shows that these groups branch and anastomose freely. Owing to this, connection is established between the individual groups of both the inner and the outer phloem. A similar

connection occurs between the outer and the inner phloem through the leaf and branch gaps (Pl. 40, B).

Through such a connection the inner and outer phloem become interdependent, just as do the individual groups of either region through branching and anastomosis, as stated above. The physiological importance of the connection of the phloem groups becomes self-evident and must be taken into account when interpreting pathological conditions of the conducting system of the plant.

Secondary growth is quite extensive, and it is necessary to understand the relation of the primary growth to secondary tissues in order to judge correctly their relative importance. It is obvious that there is a great amount of secondary wood formed, but the importance of the secondary phloem seems to have escaped attention; at least it has often been held that secondary phloem does not play a great rôle in the transport of plastic materials. But since the large amount of secondary phloem formed consists chiefly of sieve tubes, it seems self-evident that it is of primary importance in translocation. Before the time of tuberization the movement of plastic materials is localized, most of the organic food being used in the building up of new tissues and for respiration. For the comparatively small downward movement the primary phloem is sufficient, and little or no secondary phloem develops. At the time of flowering, when tuber formation is under way, secondary sieve tubes are formed in large numbers. But while the secondary phloem is formed and takes part in the translocation of plastic materials, the primary groups remain active until the plant is mature. Of course, the delicate walls of the phloem elements become somewhat thickened, but this is a condition to be expected in older structures.

The process of tuber formation has been treated by Reed, whose observations this study confirms and extends. But while Reed believes most of the tuber tissue to be formed by the pith and the perimedullary zone, the writer is led to conclude that the pith does not contribute much to the formation of new tissues, but that it is especially the perimedullary zone which forms most of the tuber. There is further a divergence of opinion in regard to the origin of the periderm. De Vries (*7*, *8*), states that the periderm is formed by the epidermis, whereas Reed (*11*) shows figures to prove that it arises from the hypodermis. A study of the series of photographs (Pl. 39, A–F) shows that, though most of the periderm is formed from the hypodermis, a superficial periderm several cells thick is formed by the epidermis.

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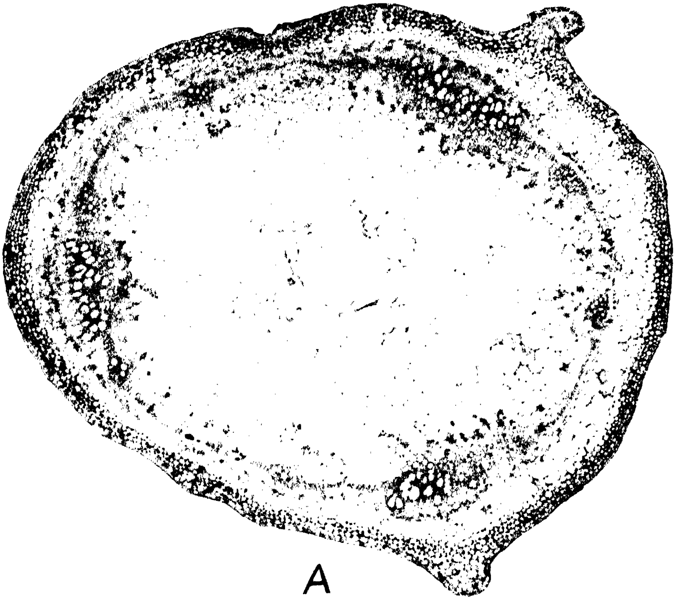
PLATE 27

Origin of leaf and branch traces of the potato:

A.—Transverse section through internode of a partly mature stem. $\times 13$.

B.—Transverse section through a stem immediately below a node, showing the origin of a new trace at *a*. $\times 13$.

(See also Plate 28.)



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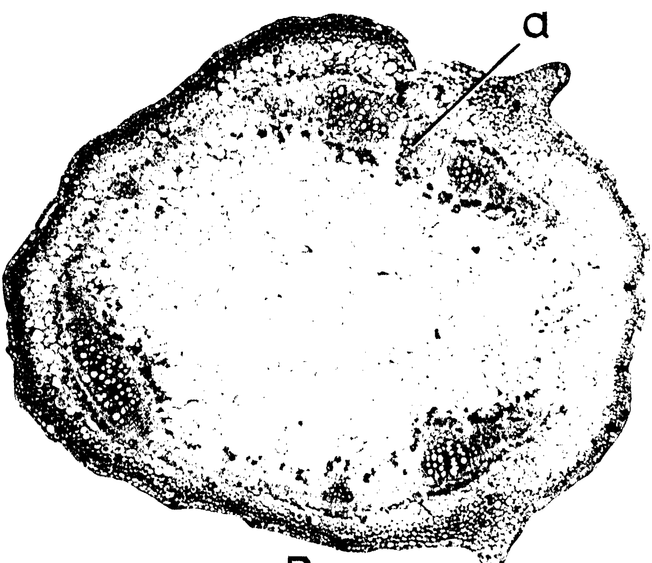

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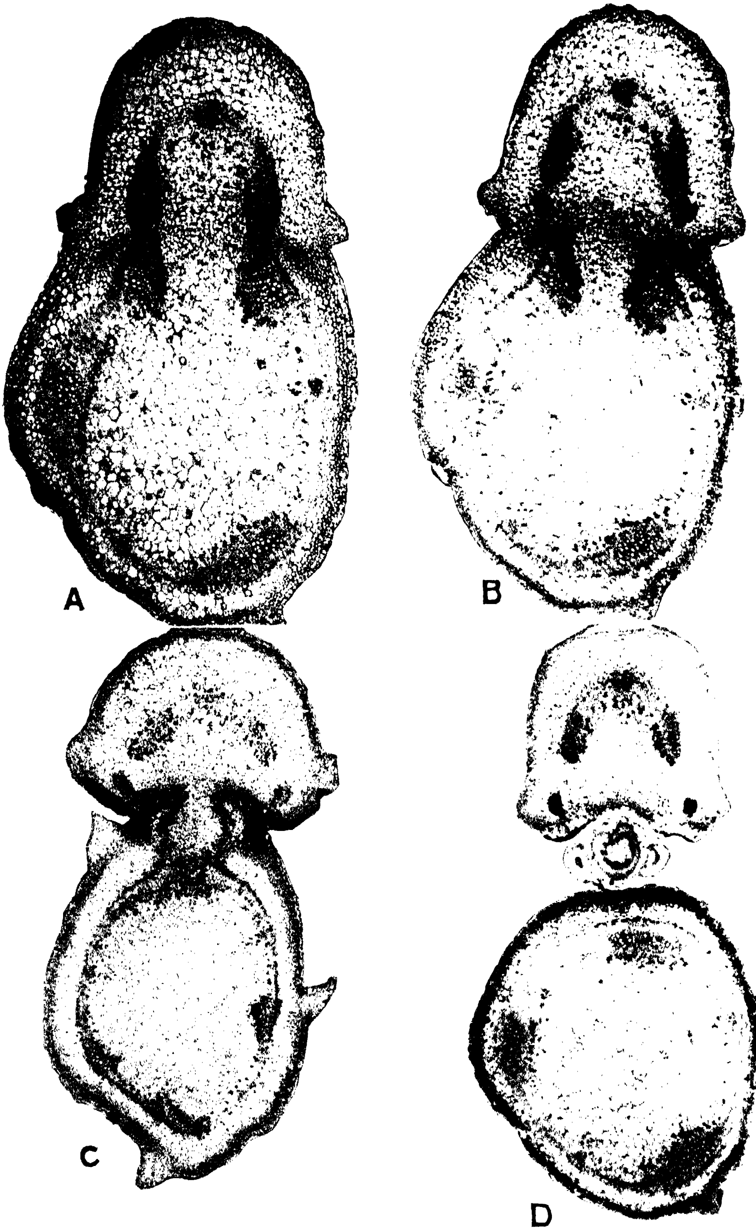


PLATE 28

Origin of leaf and branch traces of the potato:

A–D.—Transverse sections through successively higher nodal regions, showing the origin of the lateral leaf traces and of the branch trace. $\times 9$.

(See also Plate 27.)

PLATE 29

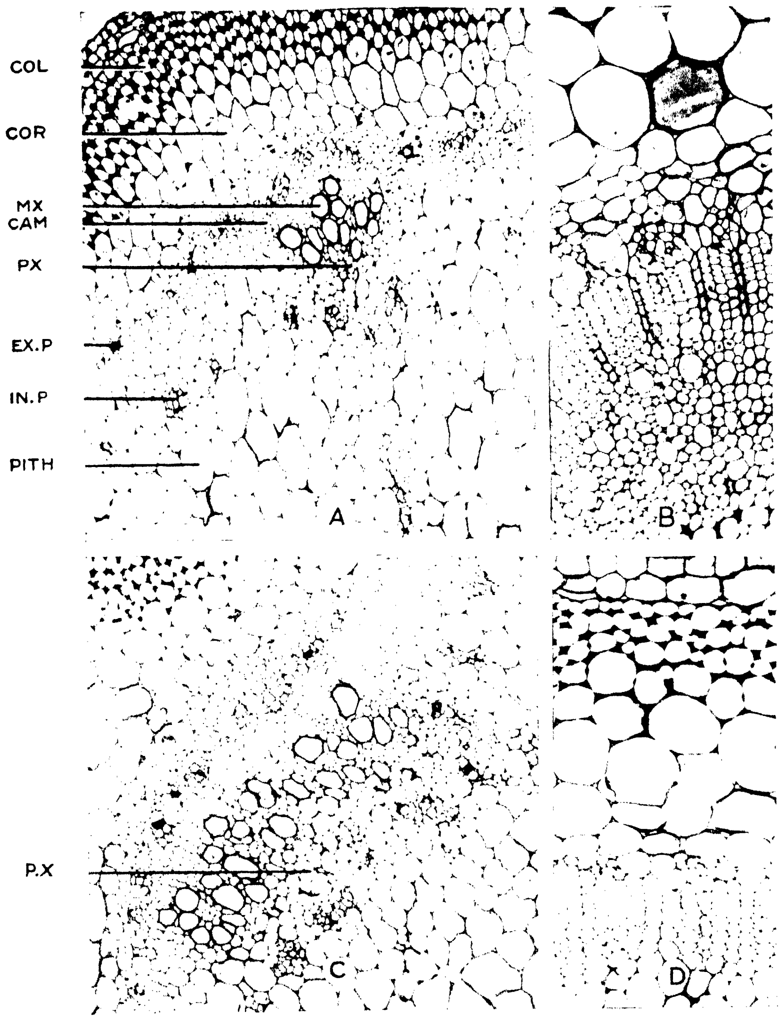
Distribution of tissues (primary) in the potato:

A.—Transverse section of part of the central cylinder and the cortex, showing small stem bundle, extent and position of external and internal phloem, proto- and meta-xylem, collenchyma and cortex, cambium, and pith. X94.

B.—Transverse section through part of large stem bundle showing fascicular cambium and medullary rays.

C.—Transverse section through a large stem bundle. X94.

D.—Transverse section through somewhat older stem, showing interfascicular cambium and position of external phloem groups and endodermis.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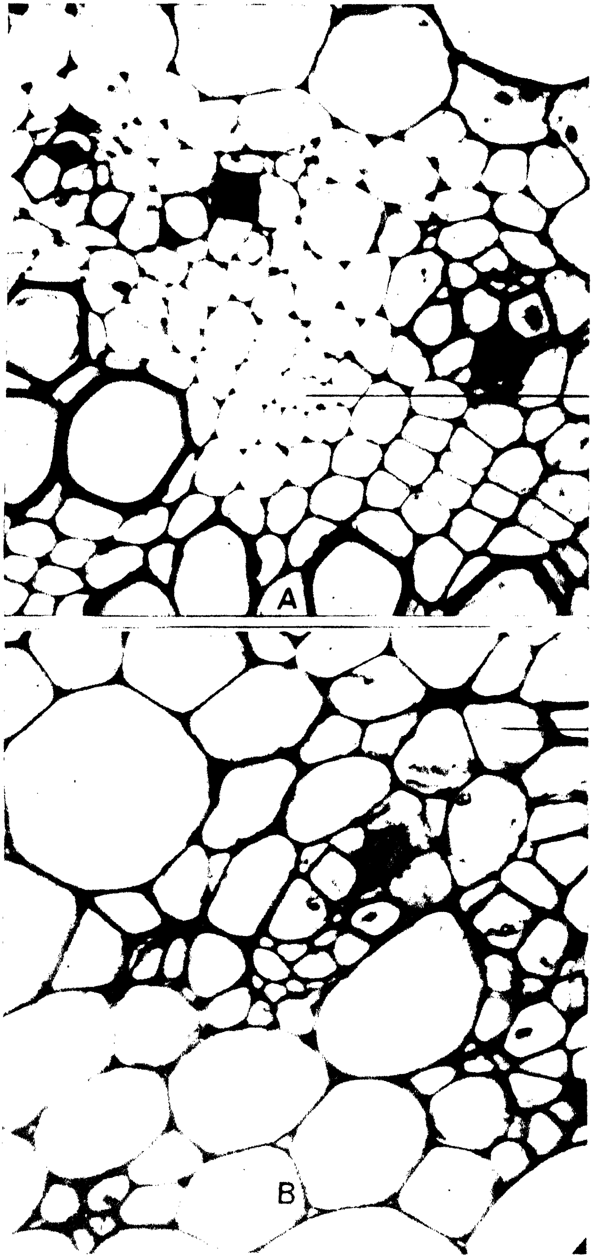

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PLATE 30

Types of primary tissues of the potato:

A.—Transverse section of part of large stem bundle showing sieve tubes and companion cells in outer phloem, type of cambium and medullary ray initials, metaxylem. ×500.

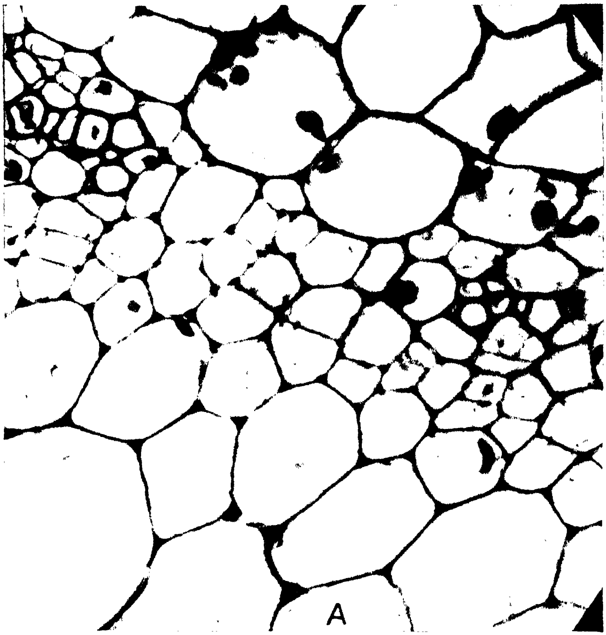
B.—Transverse section through part of the same bundle showing inner phloem, pith, and cells of the perimedullary zone. ×500.

PLATE 31

Types of primary tissues and elements of the potato:

A.—Transverse section through interfascicular region of central cylinder showing cambium, outer phloem groups, and endodermis. ×500.

B.—Transverse section through the same region showing internal phloem groups. ×500.



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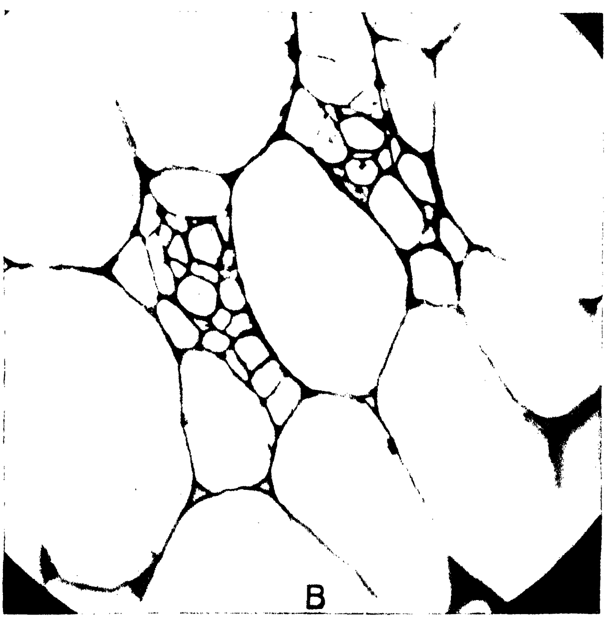

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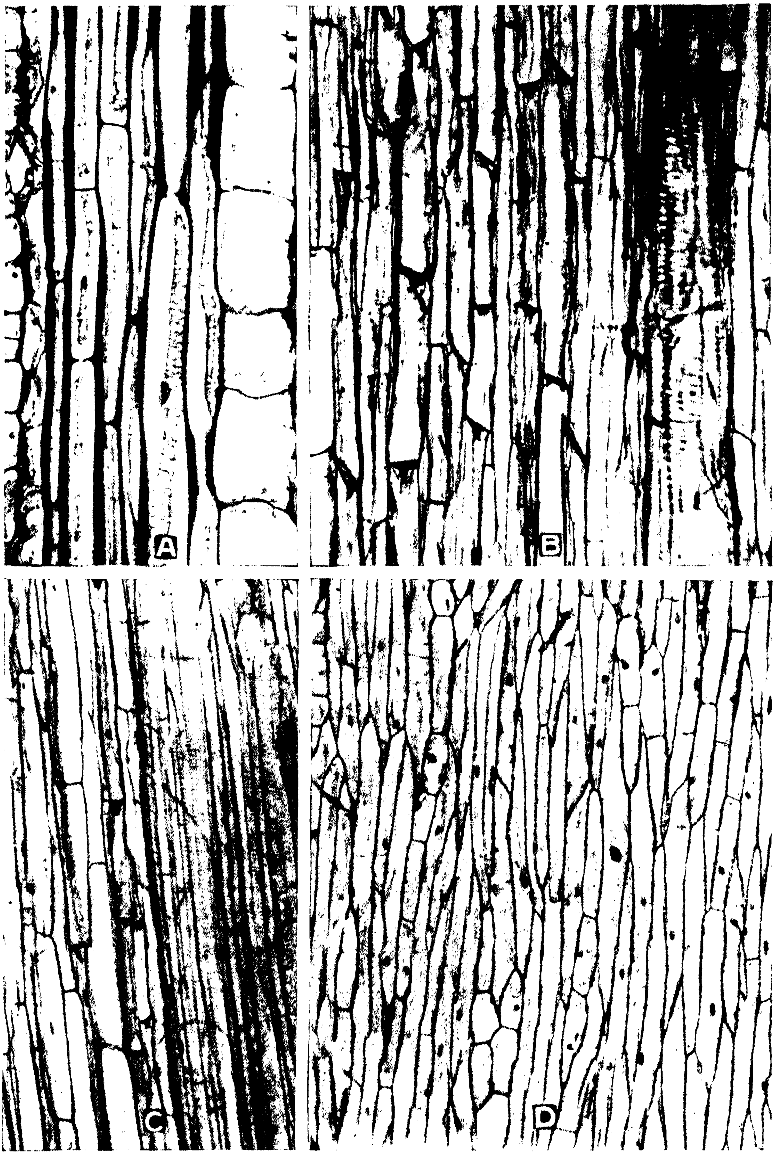
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PLATE 32

Types of elements of the potato:

A.—Radial section of outer cortex, collenchyma, subepidermal layer, and epimeris with stomata. X109.

B.—Tangential section of part of vascular cylinder of partly mature stolon showing numerous sieve tubes and two porous vessels. X218.

C.—Tangential section of young stem showing sieve tubes of internal phloem and protoxylem. X109.

D.—Tangential section of partly mature stem showing cambium and medullary ray initials. X104.

PLATE 33

Distribution of tissues and type of elements of the potato:

A.—Transverse section of large stem bundle at time of secondary growth showing distribution and type of medullary rays. ×103.

B.—Transverse view of sieve plate of secondary sieve tube. ×405.

C.—Transverse section through a large stem bundle showing type of cells of perimedullary zone and the extent of the latter. ×103.

D.—Transverse view of sieve plate greatly enlarged. ×90.

E.—Radial section through porous vessel, showing type of end wall and extent of pitting. ×292.


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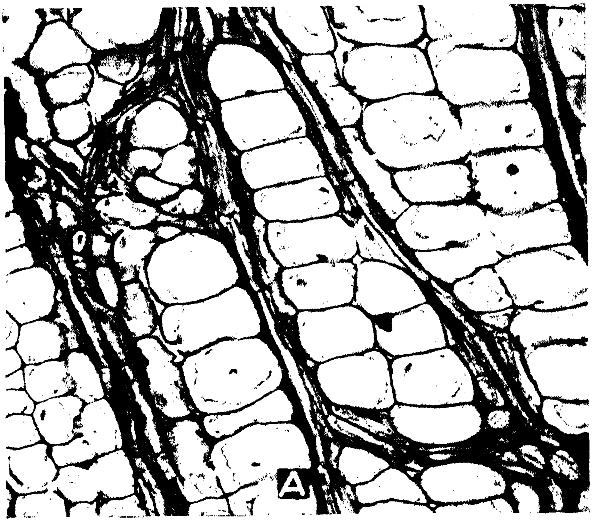
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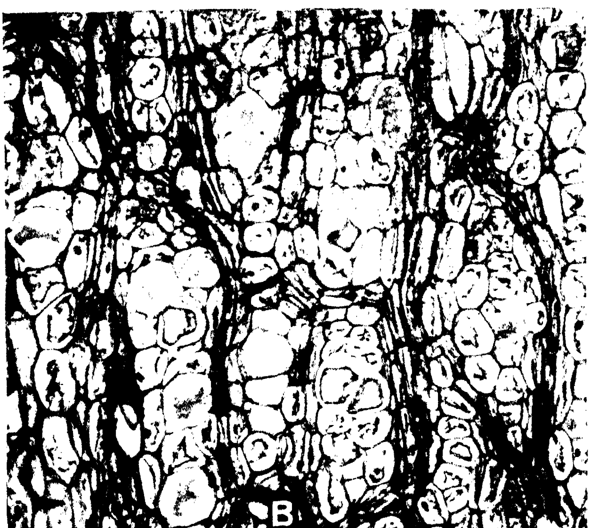
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PLATE 34

Distribution of tissues of the potato:

A.—Tangential section through external phloem, showing branching and anastomosing of phloem groups. X118.

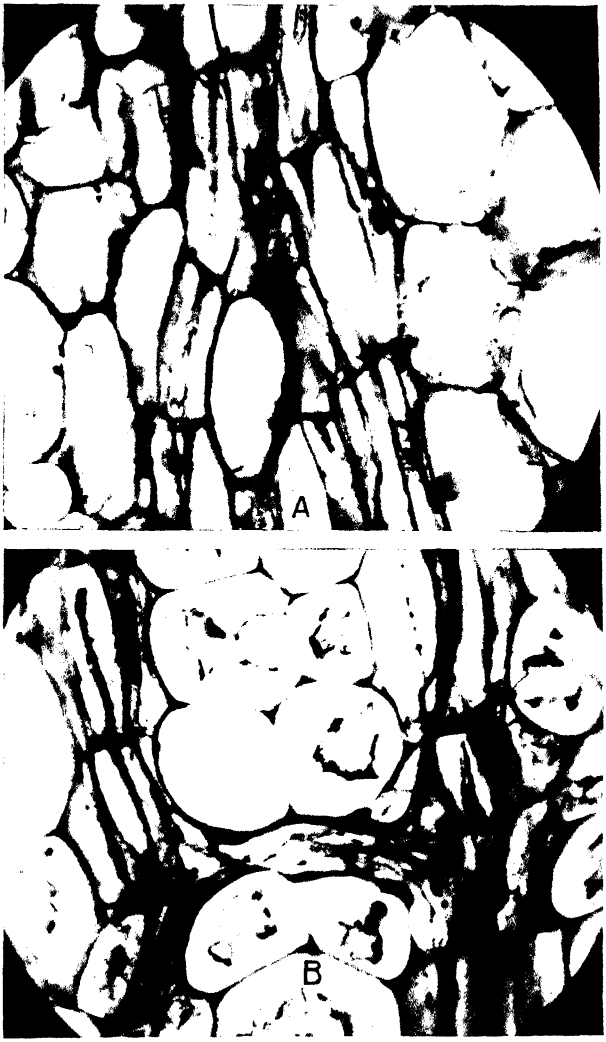
B.—Tangential section through internal phloem, showing branching and anastomosing of phloem groups. X112.

PLATE 35

Types and anastomosis of sieve tube of the potato:

A.—Enlarged view of part of Plate 34, B, showing type of sieve tube. ×400.

B.—Enlarged view through another region of the same figure, showing type and anastomosing of sieve tubes. ×400.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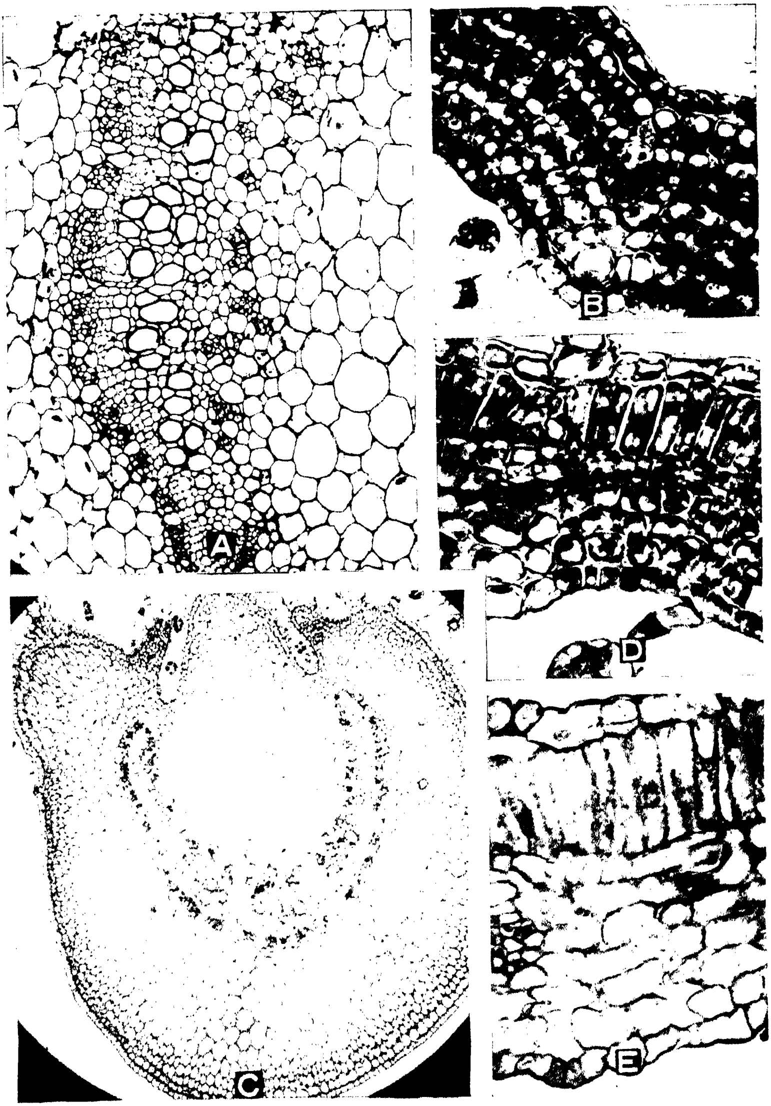
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PLATE 36

Distribution of tissues of the potato:

A.—Transverse section of lateral bundle of mature petiole, showing distribution of external and internal phloem and amount and arrangement of xylem. × 396.

B, D, E.—Cross sections through leaf blade at different stages of development, showing differentiation of palisade layer and spongy parenchyma. × 369, 342, 405, respectively.

C.—Transverse section of petiole, showing arrangement of vascular tissue, amount of cortex, and distribution of collenchyma. × 42.

PLATE 37

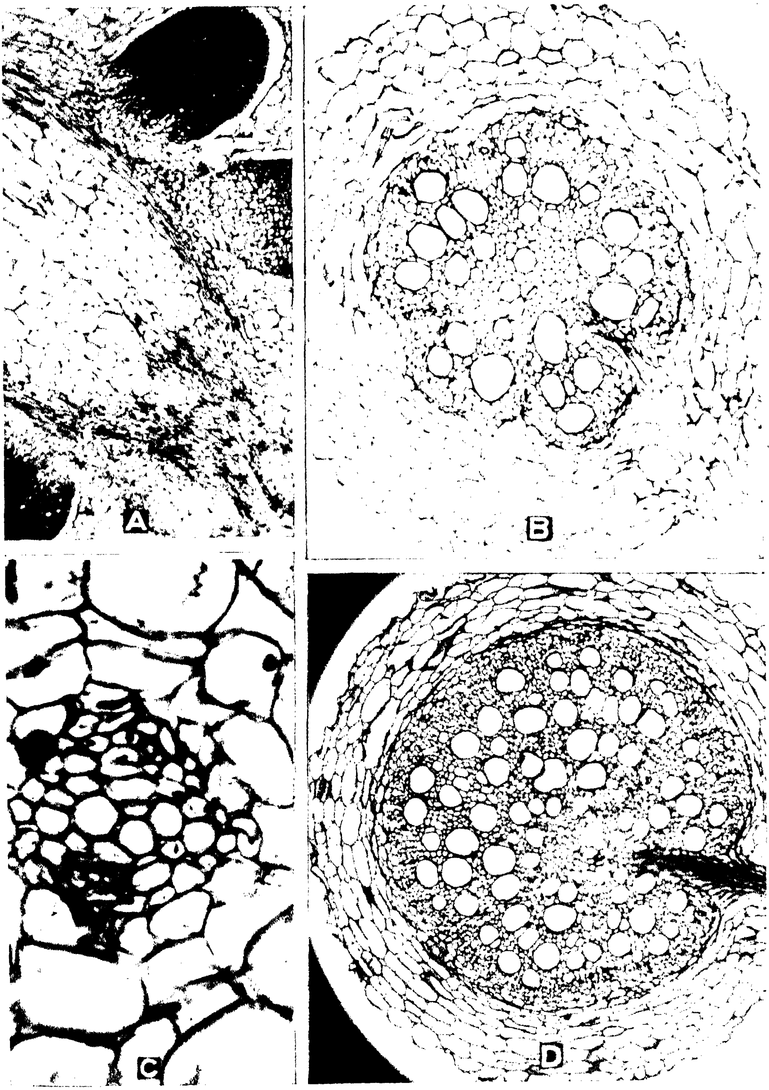
Root of the potato in development:

A.—Radial section through nodal region of underground part of stem, showing origin of roots from the pericycle. × 36.

B.—Transverse section of partly mature root. × 90.

C.—Transverse section of a young diarch rootlet, showing arrangement of protoxylem and protophloem. × 648.

D.—Transverse section of fully mature root. × 54.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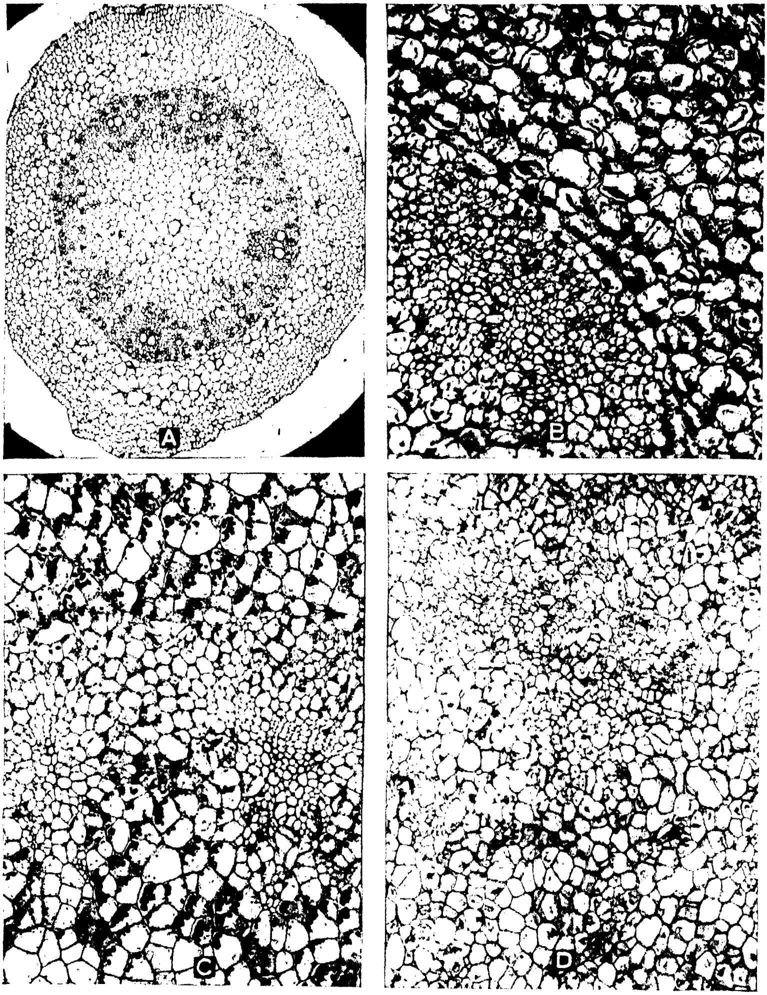
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PLATE 38

Development of the tuber of the potato:

A.—Section of mature stolon, showing general distribution and relative proportions of tissues. $\times$ 30.

B.—Transverse section of part of vascular tissue of young tuber and cortex. $\times$ 63.

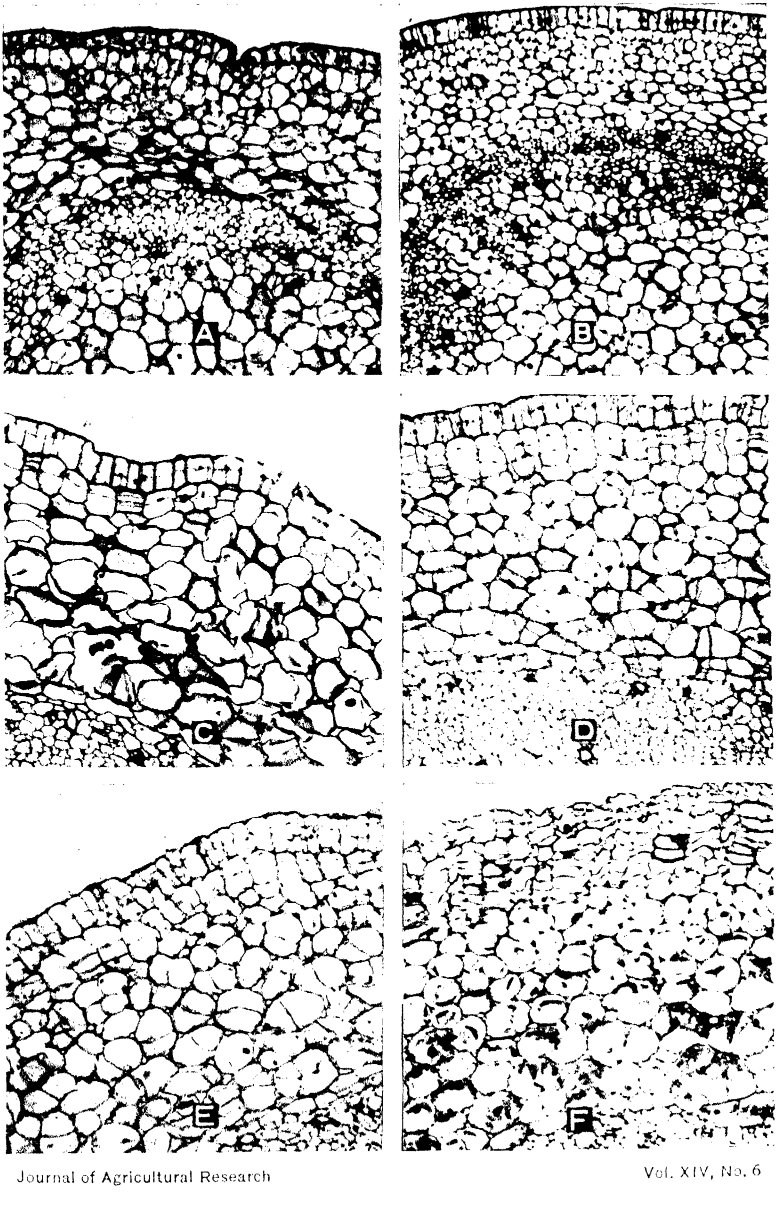
C.—Transverse section of part of vascular tissue of a somewhat older tuber, showing the beginning of extensive cell division in perimedullary zone and parenchyma of outer phloem. $\times$ 63.

D.—Transverse section of vascular tissue of partly grown tuber, showing the distribution of the phloem groups after a period of extensive growth in the perimedullary zone. $\times$ 63.

PLATE 39

Development of the tuber of the potato:

A–F.—Transverse sections through parts of tubers at successive stages of development, showing origin and development of the periderm. $\times$ 99.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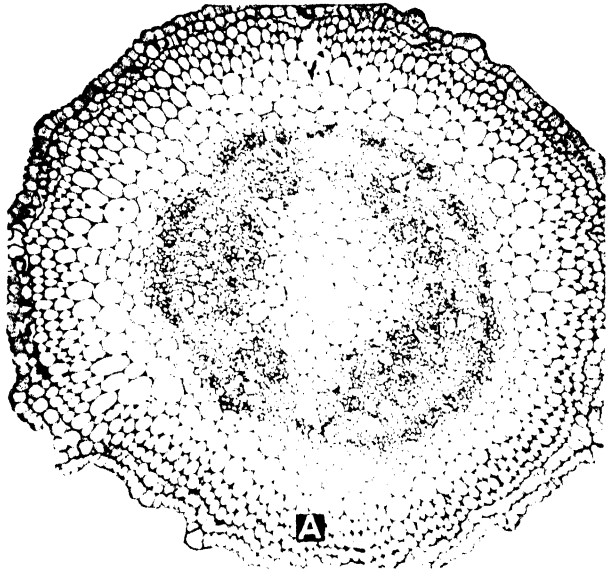


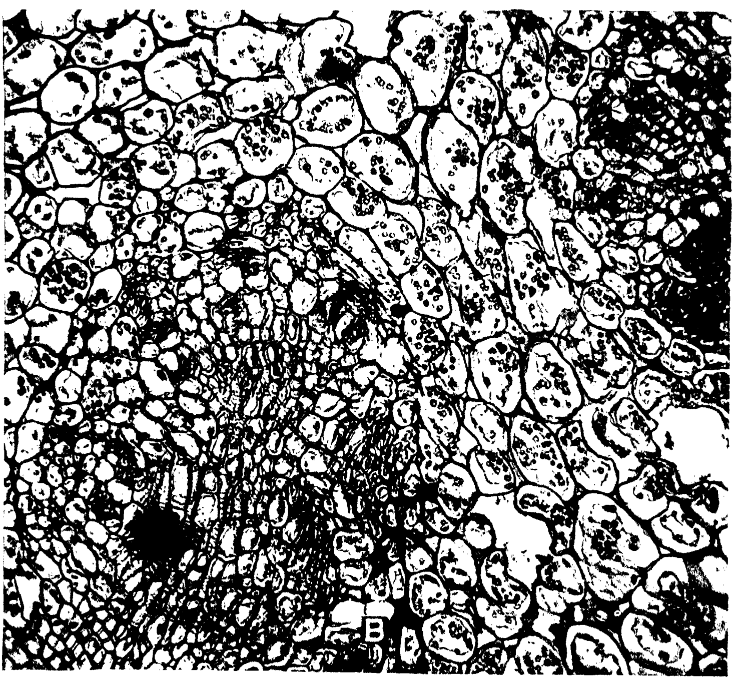


PLATE 40

Flower pedicel and stem node of the potato:

A.—Transverse section through pedicel of flower. $\times$ 46.

B.—Portion of transverse section through node, showing part of wing bundle of petiole above, part of stem bundle below, and leaf gap in center with connection of inner and outer phloem along the side of the stem bundle. $\times$ 180.

PLATE 41

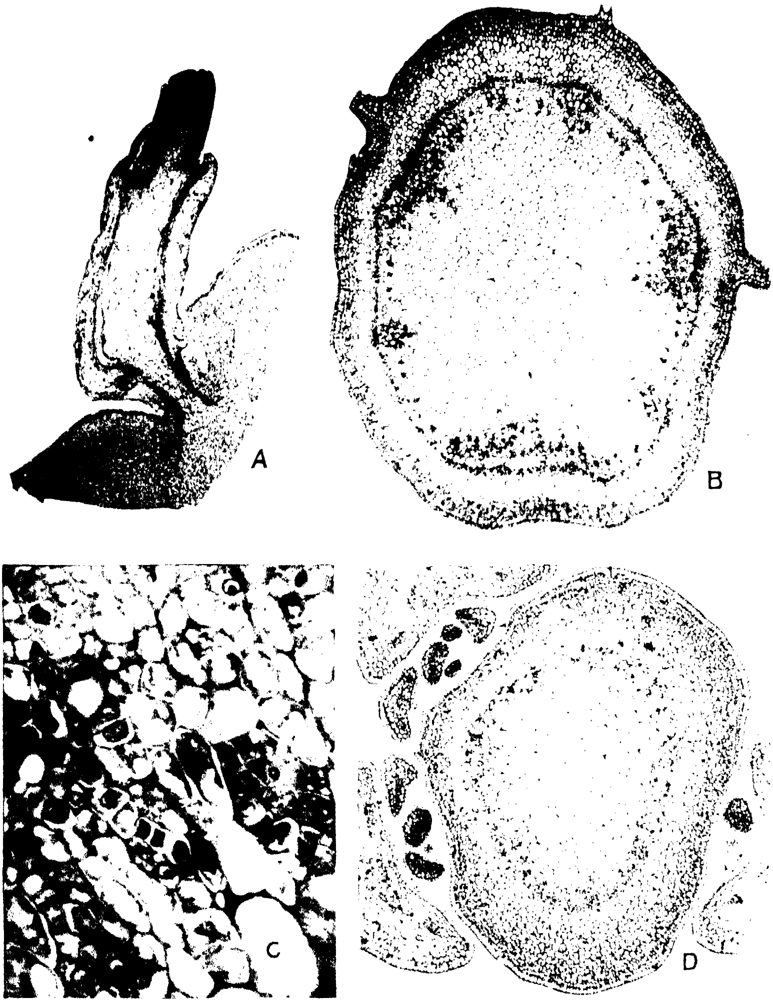
Ontogeny of the potato:

A.—Radial section of potato eye and part of mother tuber showing amount and position of procambium. × 5.4.

B.—Transverse section through tip of potato stem, showing the general distribution of tissues, the amount of vascular tissue, and its arrangement into groups. × 39.

C.—Transverse section through growing region of potato eye, showing the first visible differentiation of internal phloem groups. × 414.

D.—Transverse section through tip of potato eye, showing the arrangement of the various parts and the beginning of vascular differentiation. × 22.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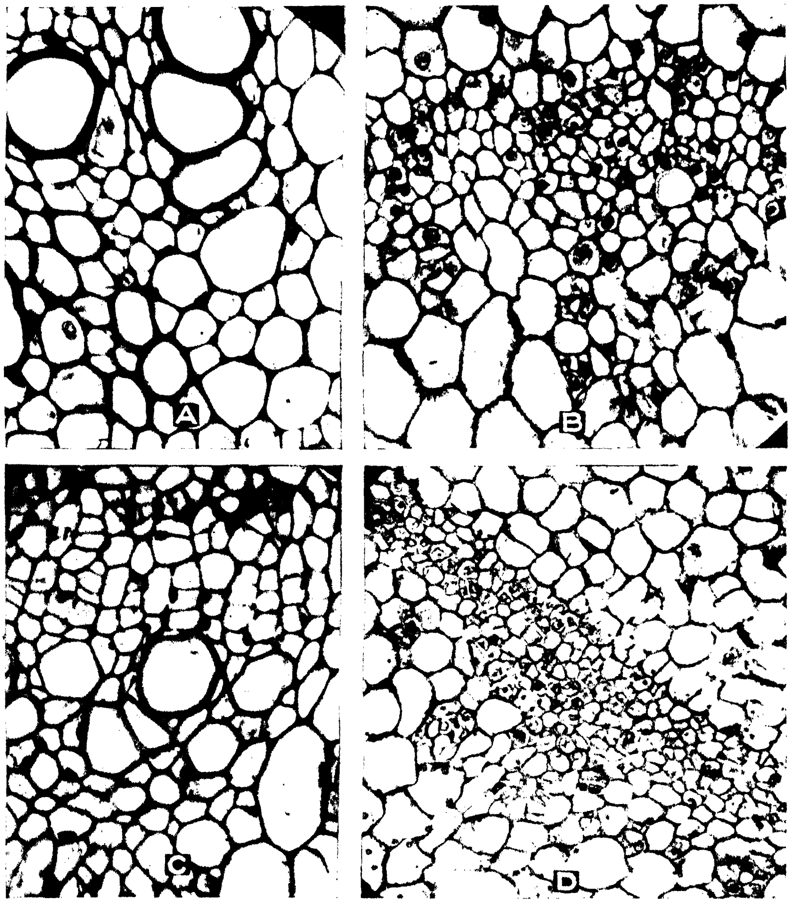
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PLATE 42

Ontogeny of the potato:

A.—Transverse section through potato sprout showing metaxylem and primary medullary rays. × 450.

B.—Transverse section through part of growing region of potato sprout, showing position of the first formed protoxylem and the differentiation of the internal and external phloem groups from the procambium. × 405.

C.—Transverse section through potato sprout, showing beginning of cambium development. × 396.

D.—Transverse section through distal region of potato sprout, showing the first differentiation of internal phloem and protoxylem (X). × 342.

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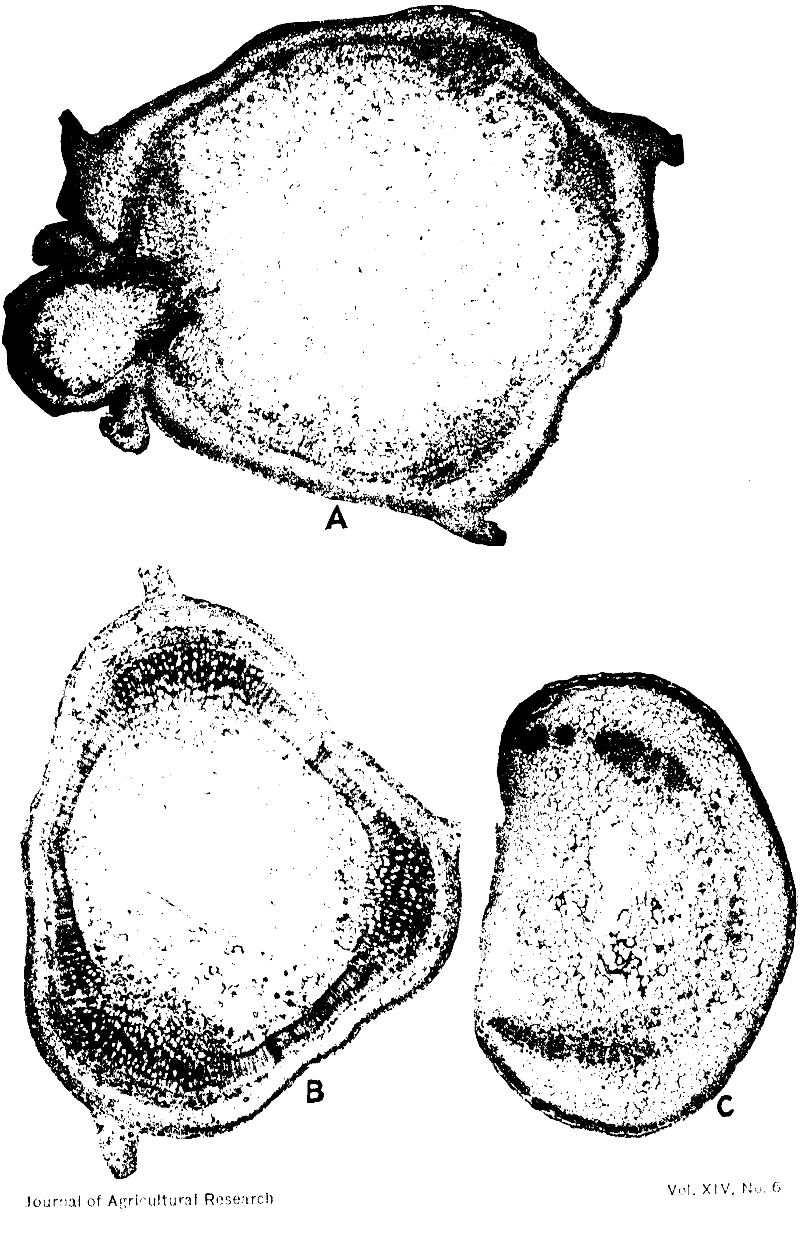
PLATE 43

Secondary growth of the potato:

A.—Transverse section through nodal region of mature stem. $\times$ 6.
B.—Transverse section through internode of mature stem. $\times$ 9.
C.—Transverse section through mature petiole. $\times$ 9.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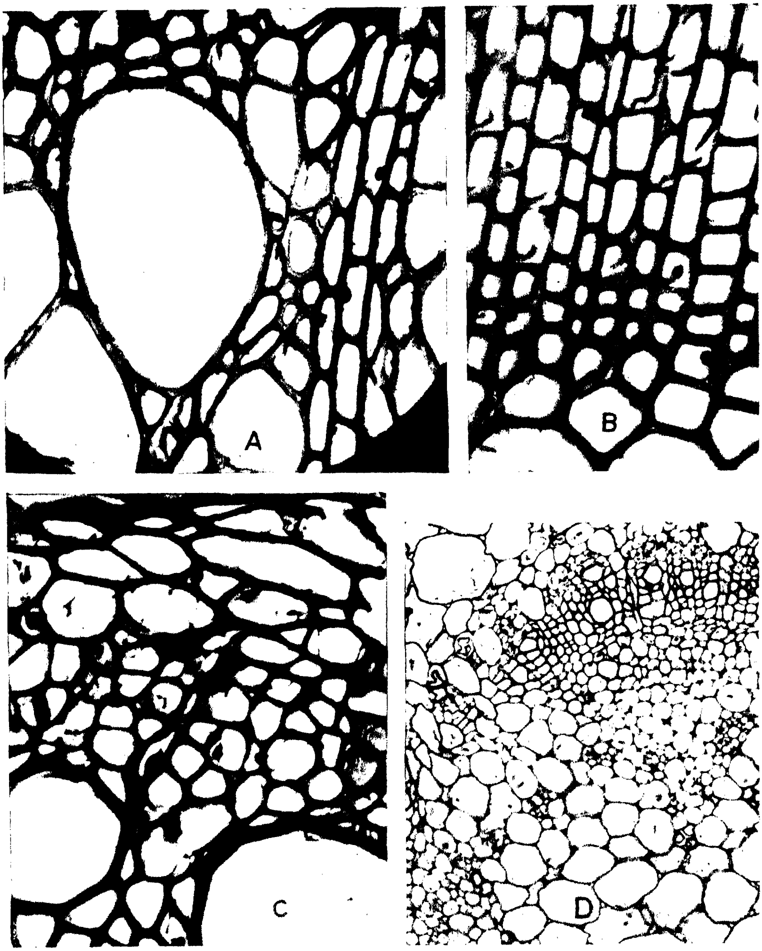
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PLATE 44

Secondary growth of the potato:

A.—Transverse section through part of large stem bundle, showing type of xylem and medullary ray cells. × 234.

B.—Transverse section through interfascicular region of mature stem, showing the types of first and later formed secondary xylem elements. × 234.

C.—Transverse section through part of mature root, showing secondary phloem. × 540.

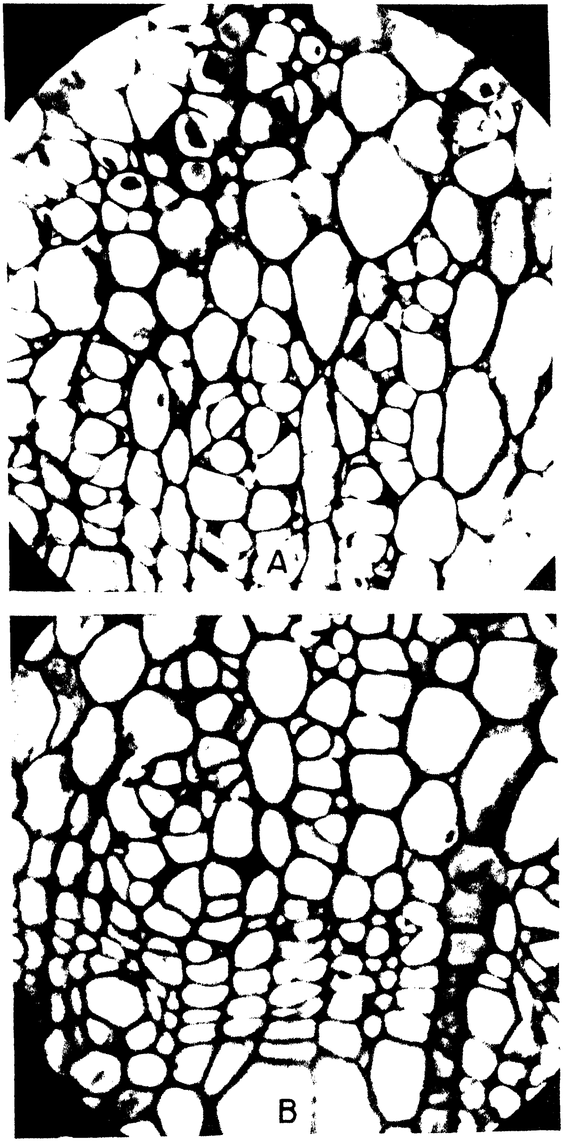
D.—Transverse section through part of mature petiole, showing secondary xylem. × 360.

PLATE 45

Secondary growth of the potato:

A.—Transverse section through phloem of mature stem, showing most of the secondary elements to be sieve tubes and rays. $\times$ 400.

B.—Transverse section through another region of mature stem, showing secondary phloem and rays. $\times$ 400.



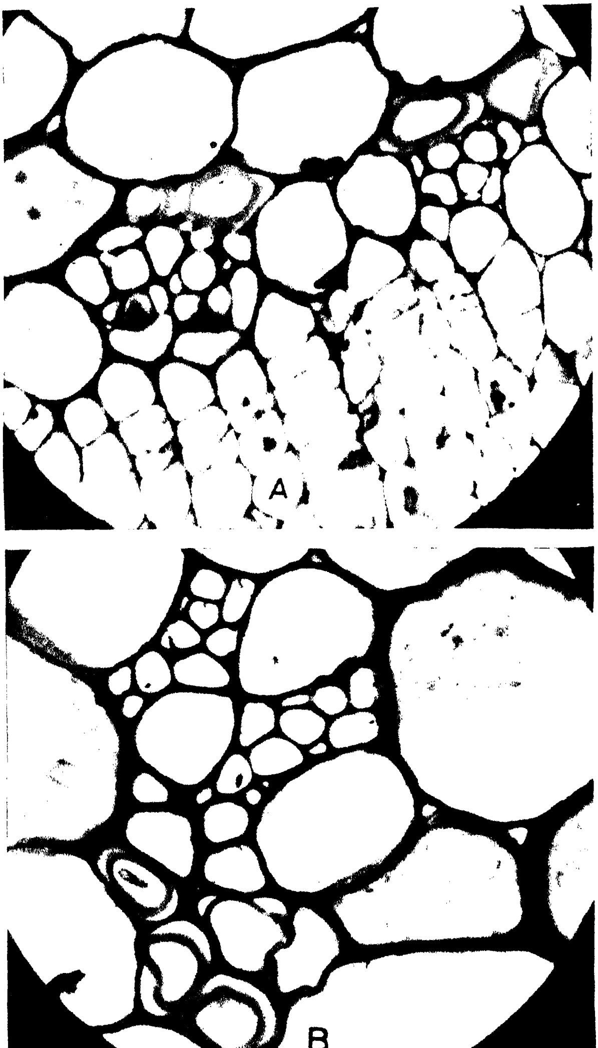

A
B

PLATE 46

Condition of the primary phloem in mature stems of the potato:

A.—Transverse sections through mature stem, showing primary phloem groups in interfascicular region. × 400.

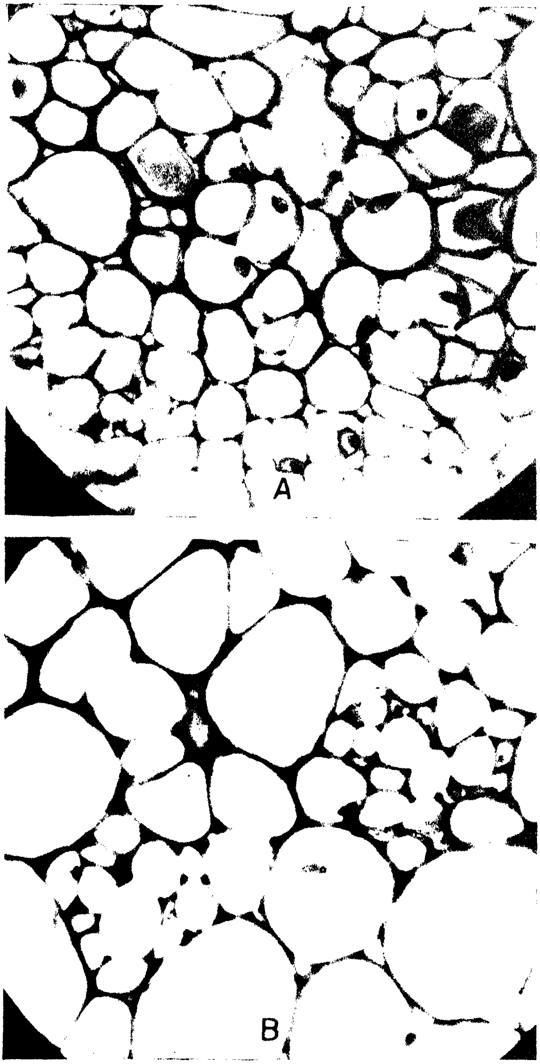
B.—Transverse section through the same region, showing primary internal phloem. × 400.

PLATE 47

Condition of primary phloem in mature stems of the potato:

A.—Transverse section showing that most of the secondary phloem is made up of sieve tubes and ray cells. × 400.

B.—Transverse section through mature stem, showing large internal phloem groups. × 400.


A
B

IMPROVED METHODS OF IMMUNIZATION AGAINST SYMPTOMATIC ANTHRAX (BLACKLEG)

By R. A. Kelser

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INTRODUCTION

During the past several years exceptional interest has been manifested by various investigators in the United States in prophylaxis against symptomatic anthrax. Up to that time the method of immunization practically exclusively employed in this country for the prevention of the disease was that of Kitt's, or a modification of his method.

Since 1897 the Bureau of Animal Industry of the United States Department of Agriculture has prepared and distributed to stock owners throughout the country millions of doses of blackleg vaccine, employing in its preparation the method outlined by Kitt with some modifications. The drinciple of the method[1] lies in the attenuation of affected muscle tissue from animals that had died of blackleg, and is accomplished by subjecting the same to a temperature of 95 to 96° C. for a period of six hours. The finished product consists of finely powdered muscle tissue containing the attenuated organisms. The results obtained from the use of this vaccine have been very satisfactory, and its use has been a leading factor in the control of the disease in the United States and elsewhere.

At the present time, however, there are, of the more recently introduced products, two which bid fair to surpass in efficacy the various other agents for immunization against blackleg. One of these represents a so-called "germ-free vaccine" or "natural aggressin," and is a sterile filtrate prepared from affected animal tissues. The other is a toxic culture filtrate and is prepared from cultures of the bacillus of symptomatic anthrax produced with special culture media for toxin production and subsequently rendered free from organisms by filtration through bacteria-retaining filters.

Both of these biological products have been found to possess valuable immunizing properties against blackleg, and their methods of preparation are such that the finished products are superior to the powdered vaccine.

[1] For details of this method see Nörgaard, V. A. Blackleg: its nature, cause, and prevention. U. S. Dept. Agr. Bur. Animal Indus. Circ. 31, 23 p. 1900.

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Numerous tests and experiments have been conducted with both agents, but from the standpoint of production of the two products the efforts of the writer have been concentrated principally on the preparation of the toxic culture filtrate.

GERM-FREE VACCINE OR NATURAL AGGRESSIN

Based on Bail's [1] work on aggressins, Schöbl [2] tried and was able to immunize calves and guinea pigs against blackleg by vaccinating them with sterilized edematous fluid from animals which had died of the disease, the sterilization of the edematous fluid being accomplished through treatment with toluol.

Franklin and Haslam [3] conducted numerous experiments with an immunizing agent based on this same principle and emphasized its value in the prevention of blackleg. The writer has prepared one lot of such a product and has tested with very satisfactory results a number of specimens of similar material received from various outside sources. The following procedure is followed in preparing the product.

Susceptible animals are inoculated intramuscularly with an emulsion prepared from the affected muscle tissue of animals dead of blackleg. The animals usually succumb to the disease in from 36 to 48 hours. The skin is then removed, and the edematous fluid from the affected area and the affected muscle tissue is collected. The tissue is then finely ground and together with the edematous fluid collected is placed in fruit jars and is frozen, an ice-salt mixture being used. After several hours' freezing the jars are removed and inverted over funnels containing thin films of cotton, the funnels all draining into a pan which converges to the center, from which by means of a spout the thawed fluid is discharged into a bottle. Ice is kept packed around the bottle in order to keep the fluid at a low temperature, as the process of thawing requires considerable time even in warm weather. This freezing process is necessary to facilitate filtering. After the dripping from the jars ceases, the "clots" are pressed to extract more of the fluid, but the material thus obtained is kept separate from the other fluid and is filtered last, as it passes through considerably slower and tends to clog the apparatus. The product is filtered twice through Berkefeld filters (first through one of "V" and then through one of "N" porosity), is preserved with chloroform (0.5 per cent), and is ready for testing.

[1] BAIL, Oskar. VERGLEICHENDE UNTERSUCHUNGEN ÜBER MILZBRANDFEINDLICHE EIGENSCHAFTEN IN ORGANISMUS DES HUNDES UND KANINCHENS. *In* Centbl. Bakt. [etc.], Abt. 1, Bd. 27, No. 1, p. 10–21. 1900.
——— UNTERSUCHUNGEN ÜBER NATÜRLICHE UND KÜNSTLICHE MILZBRANDIMMUNITÄT. *In* Centbl. Bakt. [etc.], Abt. 1, Bd. 33, No. 5, p. 343–353. 1903.

[2] SCHÖBL, Otto. WEITERE VERSUCHE ÜBER AGGRESSINIMMUNISIERUNG GEGEN RAUSCHBRAND. *In* Centbl. Bakt. [etc.], Abt. 1, Bd. 62, No. 3/4, p. 296–304. 1912.

[3] FRANKLIN, O. M., and HASLAM, T. P. THE STRENGTH AND COMPOSITION OF BLACKLEG VACCINES. *In* Jour. Infect. Diseases, v. 19, no. 3, p. 408–415. 1916.

POTENCY TESTS OF THE GERM-FREE VACCINE OR NATURAL AGGRESSIN

A number of tests have been carried out on calves and guinea pigs to determine the potency of this biological product and the results obtained have been very satisfactory. The samples tested were from various sources, the greater part of them being submitted by commercial houses in connection with applications for licenses to market the product interstate.

The early tests of this product on guinea pigs were not nearly so satisfactory as the tests on calves, owing in a large measure to the character of the virus employed for the subsequent infection of the vaccinated guinea pigs. This test, however, has been much improved through the use of a different type of virus which is described on page 260.

The results of all the tests carried out with this product will not be tabulated, but the following three tests are given as examples of the results uniformly obtained (Tables I–III).

TABLE I.—*Results of subcutaneous inoculation of guinea pigs with germ-free vaccine followed after 14 days by intramuscular inoculation with blackleg virus*

Guinea-pig No.	Amount of vaccine.	Amount of virus.[a]	Result.
	Cc.	*Cc.*	
1	1	0.5	Dead of blackleg in 24 hours.
2	1	.5	Marked swelling but remained alive.
3	2	.5	Dead of blackleg in 30 hours.
4	2	.5	Dead of blackleg in 48 hours.
5	3	.5	Slight swelling, remained alive.
6	3	.5	Do.
7	4	.5	Dead of blackleg in 36 hours.
8	4	.5	Remained alive.
9	Control	.5	Dead of blackleg in 24 hours.
10	...do...	.5	Dead of blackleg in 32 hours.

[a] The virus employed in this instance was prepared by emulsifying 10 gm. of ground affected muscle tissue from a calf dead of blackleg with 30 cc. of physiological salt solution, and filtering through a thin film of cotton.

TABLE II.—*Results of subcutaneous vaccination of calves followed after 14 days with intramuscular inoculation of blackleg virus*

Calf No.	Amount of vaccine.	Amount of virus.[a]	Result.
	Cc.	*Cc.*	
1	5	10	Remained alive.
2	5	10	Do.
3	Control	10	Died of blackleg.
4	...do...	10	Do.
5	...do...	10	Do.
6	...do...	10	Do.

[a] The virus consisted of a heavy suspension of emulsified affected muscle tissue.

The use of a relatively large number of controls in the calf inoculation tests was due to the fact that these experiments were made at times when calves were to be infected for use in the preparation of the regular blackleg vaccine for distribution by the Bureau of Animal Industry. At such times four to six calves are usually infected; therefore in order to add to the value of the test four such animals as controls were employed.

TABLE III.—*Results of subcutaneous vaccination of calves followed after 5½ months with intramuscular injections of blackleg virus*

Calf No.	Amount of vaccine.	Amount of virus.[a]	Result.
	Cc.	*Cc.*	
1.....	5.......	10	Remained alive.
2.....	5.......	10	Do.
3.....	Control.	10	Died of blackleg.
4.....	...do....	10	Do.
5.....	...do....	10	Do.
6.....	...do....	10	Do.

[a] Virus employed of same character as described in Table II.

PREPARATION OF THE TOXIC CULTURE FILTRATE

Leclainche and Vallée[1] and others have demonstrated that the bacillus of symptomatic anthrax when grown under favorable conditions produces a true toxin. It has also been demonstrated that animals susceptible to blackleg could be effectively immunized against the disease by injecting them with small amounts of such toxin-containing filtrates. Considerable attention has been paid to this method in Japan by Nita,[2] and in this country Eichhorn [3] has recently called attention to it.

It was at first thought that this toxin was of a very stable nature and not materially affected by such influences as air, light, moderate degrees of heat, drying, etc. Subsequent investigations, however, have shown that such is not the case, but, on the contrary, under various conditions, it is more or less unstable. Therefore, in the production of this toxin this fact must be borne in mind, and precautions taken throughout the process to guard against influences likely to affect the material.

Numerous types of media have been prepared and tested for the production of this toxin, and considerable difference has been found in the potency obtained with the various kinds of media. A description will be given only of the type of medium the writer considers most efficient for toxin production, but the following are other medias tried: Dextrose-veal bouillon plus cubes of beef, dextrose-veal bouillon

[1] LECLAINCHE, E., and VALLÉE, H. RECHERCHES EXPÉRIMENTALES SUR LE CHARBON SYMPTOMATIQUE. *In* Ann. Inst. Pasteur, année 14, no. 4, p. 202–223. 1900.

[2] NITA. UNPUBLISHED RESULTS.

[3] EICHHORN, Adolph. STUDIES IN BLACKLEG IMMUNIZATION WITH SPECIAL REFERENCE TO BLACKLEG FILTRATE. *In* Jour. Amer. Vet. Med. Assoc., v. 52 (n. s., v. 5), no. 6, p. 653–669. 1918. Discussion, p. 663–669.

plus sterile bovine serum, dextrose-liver bouillon, dextrose-liver bouillon plus cubes of beef, dextrose-veal bouillon plus calcium lactate, dextrose-liver bouillon plus calcium lactate.

The medium with which best results were obtained is a modification of Martin's peptone bouillon, and is prepared as follows:

Fresh pig stomachs with their contents are obtained, and after trimming away the fat, are opened and the contents expelled. They are then lightly rinsed in water, care being taken not to wash away the gastric mucosa. The material is then cut in pieces of suitable size for a meat-chopping machine and finely ground. To every 200 gm. of this finely ground stomach tissue are added 1 liter of water at 50° C. and 20 cc. of hydrochloric acid, C. P. This mixture should be made in glass flasks, and from here on up to the time the material is neutralized it should not come in contact with metal. The mixture is placed in an incubator maintained at approximately 50° and allowed to remain there for 20 to 24 hours. It is then filtered through several thicknesses of cheesecloth, heated to 80° to stop peptonization, allowed to cool down to 70°, and neutralized to litmus. Flocculation occurs at this point, and the material is then filtered through cotton. Sterilization is accomplished by autoclaving at 15 pounds' pressure for 20 minutes.

A piece of fresh beef is then obtained, and with as much precaution as possible to prevent undue contamination, a thin layer is removed, taking all of the exposed surface of the beef.[1] As much of the remainder as will be required is cut in small pieces and put through a meat chopper which has been previously boiled; 450 gm. of this ground beef and 10 gm. of dextrose are then added to every 1,000 cc. of the peptone solution. The flasks containing the medium are filled close to the cotton stopper in order to eliminate all the air space possible. The medium is then allowed to remain at refrigerator temperature for several hours, at the end of which time it is titrated against phenolphthalein and the reaction adjusted to +0.5, and is then sterilized by heating and maintaining it at a temperature of 65° to 70° C. for one hour on three successive days. It is then ready for inoculation.

The inoculation may be made either with a freshly isolated, virulent culture of the bacillus of symptomatic anthrax or with fresh affected muscle tissue known to contain only the blackleg organism. The latter method is not as easy of accomplishment as the first.

The culture that gives good results is one 24 to 48 hours old, recovered from a guinea pig which has been inoculated with virulent blackleg material. The cultures may be taken from the affected musculature, peritoneal fluid, or heart blood after the animal has been dead a few hours. Dextrose, beef, or liver bouillon may be employed in recovering

[1] This rejected material can be utilized in the preparation of ordinary beef bouillon.

the organism. A number of cultures should be made and care exercised to select for inoculation only a pure culture of the blackleg organism.

It has been the procedure of the writer to plan the work so that the culture would be ready for inoculation on the day the medium for toxin production was sterilized for the third time. The medium is allowed to cool down to approximately 40° C., and then several cubic centimeters of the culture are inoculated in the bottom of each of the flasks with a sterile pipette and the flasks placed in the incubator at 37.5°. If the inoculation is not made immediately following the third sterilization, the medium should be heated to 60° to drive off the oxygen and should be inoculated after it has cooled down to approximately 40°. Incubation for 10 to 12 days appears to be the approximate time for satisfactory toxin production.

The product is then removed from the incubator and filtered through several thicknesses of cheesecloth, next through a thin layer of asbestos wool, and then twice through Berkefeld filters of "N" porosity.

It is preserved with 0.5 per cent chloroform and stored in amber-glass bottles, which should be well filled, so as to leave as little air space as possible.

The product is tested culturally, and sublethal doses are administered to guinea pigs to determine whether or not it has been rendered free of organisms.

The potency of the material is determined through animal inoculation tests. In connection with the test for potency, attention has also been given to the degree of toxicity, because of the apparent relation of one to the other. This phase of the question will be given further consideration in a subsequent chapter of this article.

POTENCY TESTS OF THE TOXIC CULTURE FILTRATE

As in the case of the natural aggressin, numerous tests have been carried out with the culture filtrate on calves and guinea pigs. There is one important factor which has been uniformly noted in all the tests with the culture filtrate, and that is that there appears to be a direct ratio between the toxicity and potency of the product. In all potency tests thus far undertaken by the writer, no immunizing properties could be demonstrated in nontoxic culture filtrates. It is contemplated that if this relation of toxicity to potency is definitely proved, it will be a valuable factor in connection with standardization of the product.

Tables IV–VIII give results representative of the tests to which the filtrate has been submitted.

TABLE IV.—*Results of subcutaneous inoculation of guinea pigs* [a] *with the toxic culture filtrate, followed after 14 days with blackleg virus*

Guinea-pig No.	Amount of culture filtrate.	Amount of virus.[b]	Result.
	Cc.	*Cc.*	
1	0.25	0.5	Dead of blackleg in 36 hours.
2	.25	.5	Dead of blackleg in 48 hours.
3	.50	.5	Remained alive.
4	.50	.5	Do.
5	.75	.5	Dead of blackleg in 48 hours.
6	.75	.5	Remained alive.
7	1	....	Died from the effects of the blackleg toxin before the inoculation of the virus.
8	1	.5	Remained alive.
9	Control.	.5	Dead of blackleg in 24 hours.
10	Control.	.5	Dead of blackleg in 30 hours.

[a] Guinea pigs weighing from 320 to 380 gm. were used. The minimal lethal dose (M. L. D.) of the blackleg toxin for guinea pigs of this weight was between 1 and 1.5 cc. when administered intramuscularly.
[b] The virus employed was prepared from affected muscle tissue in the manner described under Table I.

TABLE V.—*Results of subcutaneous inoculation of guinea pigs* [a] *with toxic culture filtrate, followed after 14 days with specially prepared blackleg virus*

Guinea-pig No.	Amount of culture filtrate.	Amount of virus.	Result.
	Cc.	*Cc*	
1	0.25	0.25	Dead of blackleg in 24 hours.
2	.25	.25	Dead of blackleg in 48 hours.
3	.25	.25	Do.
4	.25	.25	Dead of blackleg in 72 hours.
5	.25	.25	Remained alive.
6	.25	.25	Do.
7	.5	.25	Dead of blackleg in 48 hours.
8	.5	.25	Remained alive.
9	.5	.25	Do.
10	.5	.25	Do.
11	.5	.25	Do.
12	.5	.25	Do.
13	Control.	.25	Dead of blackleg in 24 hours.
14	Control.	.25	Do.
15	Control.	.25	Dead of blackleg in 48 hours.

[a] Guinea pigs weighing 320 to 380 gm. were employed. The minimal lethal dose of the blackleg culture filtrate for guinea pigs of this weight was 1 to 1.5 cc. when administered intramuscularly.

In tests of various blackleg immunizing agents on guinea pigs, difficulty has been experienced by most investigators with the type of virus employed for the infection of the animal subsequent to vaccination. Most workers have employed for the purpose emulsions of affected muscle tissue. The procedure usually followed is to weigh out a definite amount of ground affected muscle tissue, emulsify with a definite amount of physiological salt solution, filter through a thin film of cotton, and use a certain quantity of the filtered solution as a test dose. It is obvious that a test virus prepared in this manner would not be very satisfactory, especially when used on small animals such as guinea pigs. The number of blackleg organisms such material would contain would undoubtedly

vary greatly with the different amounts weighed. Depending on how well the material is emulsified, a greater or smaller number of the organisms would be left behind in the small particles of tissue which are filtered out. Foreign organisms frequently present in such affected tissue also are complicating factors when injected into the guinea pigs.

The writer has prepared a type of test virus which has given very good results in the guinea-pig tests and possesses a number of advantages over the emulsion of affected tissue. It is prepared in the following manner: Guinea pigs are inoculated intramuscularly with an emulsion of virulent blackleg tissue and usually die of the disease in from 24 to 48 hours. Cultures are then made from the guinea-pig carcasses into fermentation tubes containing dextrose bouillon. The culture medium is heated for approximately 10 minutes in the Arnold sterilizer just prior to inoculation in order to drive off the oxygen. It is allowed to cool down to about 45° C., inoculated, placed in vacuum jars, and incubated 24 hours at 37.5°. At the expiration of the incubation period the jars are removed from the incubator and all fermentation tubes showing evidence of good growth removed and examined for purity. The cultures are then thoroughly mixed in a crystallizing dish with sufficient lactose to make a soft paste, and this placed in a vacuum desiccator containing sulphuric acid. Care should be taken to protect the material from direct light by covering the desiccator with towels or by keeping it in a dark place. The material dries very rapidly. It is then removed and pulverized to a very fine powder in a sterile mortar and stored in wide-mouth amber-glass bottles at refrigerator temperature. When ready for use a definite amount of the powder is weighed out and taken up in a measured amount of distilled water.

The approximate minimal lethal doses of this virus for guinea pigs weighing 350 gm. can be established and this increased 10 times for a test dose in potency tests of blackleg products.

Virus thus prepared contains no foreign organisms, eliminating the possibility of complications in the animals inoculated with it; it is readily absorbed, and can be fairly accurately standardized. In this form the virus retains its virulence for a considerable time.

TABLE VI.—*Results of subcutaneous vaccination of calves with toxic culture filtrate followed after 14 days with intramuscular inoculation of blackleg virus*

Calf No.	Amount of culture filtrate.	Amount of virus.[a]	Result.
	Cc.	*Cc.*	
1	5	10	Slight swelling. Remained alive.
2	5	10	Very slight swelling. Remained alive.
3	Control	10	Dead of blackleg within 48 hours.
4	do	10	Do.
5	do	10	Do.
6	do	10	Do.

[a] The virus employed was an emulsion of affected blackleg muscle tissue.

The following table gives the results of a test conducted with two different specimens of blackleg culture filtrate. The specimen labeled "1" was demonstrated to be nontoxic for guinea pigs, while 1.5 cc. of the sample labeled "2" would prove fatal to guinea pigs.

TABLE VII.—*Results of subcutaneous vaccination of calves with blackleg culture filtrates No. 1 and 2 followed after 14 days with intramuscular inoculation of blackleg virus*

Calf No.	Blackleg culture Filtrate No.	Amount culture filtrate.	Amount of virus.[a]	Result.
		Cc.	*Cc.*	
1	1	5	10	Dead of blackleg within 48 hours.
2	1	5	10	Do.
3	2	5	10	Very slight swelling. Remained alive.
4	2	5	10	Do.
5	Control	None.	10	Dead of blackleg within 48 hours.
6	do.	None.	10	Do.
7	do.	None.	10	Marked extensive swelling. Animal recovered.

[a] The virus employed was an emulsion of affected muscle tissue.

The following test on guinea pigs tends to demonstrate an intimate relation between the toxicity and potency of blackleg culture filtrates.

TABLE VIII.—*Results of vaccination of guinea pigs with various culture filtrates followed after 14 days with inoculation of blackleg virus*

Culture filtrate No.	Approximate M. L. D. for 350-gm guinea pigs.	Amount vaccinated in each of 3 guinea pigs.	Dose of virus injected after 14 days.	Result.
	Cc.	*Cc.*	*Cc.*	
1	Nontoxic.	1	0.5	All 3 guinea pigs died of blackleg.
X	1.5	1	.5	1 of the 3 guinea pigs died of blackleg.
Y	2.5	1	.5	Do.
3	Nontoxic.	1	.5	All 3 guinea pigs died of blackleg.
Z	Nontoxic.	1	.5	Do.
5	4	1	.5	Do.

RELATION OF THE NATURAL AGGRESSIN TO THE TOXIC CULTURE FILTRATE

The question has often arisen as to the relation between blackleg natural aggressin and the toxic culture filtrate—that is, whether or not the immunizing principles in both products are identical. While the work conducted in this respect by the writer has not as yet been sufficient to draw absolutely definite conclusions, there is one factor which points to a distinct difference in the active principles of the two products.

Of the specimens of blackleg natural aggressin tested in this laboratory comparatively large doses of the material produced no symptoms of toxemia in the guinea pigs inoculated. The same specimens, however,

were found highly efficient in immunizing experiments. In the case of the culture filtrate the toxicity of the material was readily demonstrable and its potency appears to depend on its being toxic.

It is possible, therefore, that immunization with blackleg natural aggressin is brought about through the production of "antiaggressins," while with the toxic culture filtrate immunity is acquired through the production of antitoxin.

It is highly desirable that an entirely satisfactory and practical method of concentration of the toxic culture filtrate be obtained. Some of the methods which have been employed in the past are wholly or in part unsatisfactory, the toxin being either totally destroyed or its potency considerably lowered. As the toxicity of the culture filtrate is apparently related to its potency, obtaining the toxin in a pure or concentrated form would permit the production of an accurately standardized product of uniform dosage, based on the minimal lethal dose of the toxin for guinea pigs of a given weight.

In the filtration of the toxic culture some of the toxin is lost as a result of such process. It is therefore essential that the product be subjected only to such filtration as is necessary to insure the removal of all organisms.

It is hardly necessary to emphasize the economic importance of the toxic culture filtrate as compared with the natural aggressin, since the cost of its production is only a small percentage of that necessary to prepare the natural aggressin.

CONCLUSIONS

(1) Blackleg natural aggressin and toxic culture filtrate are highly valuable agents in immunization against symptomatic anthrax.

(2) Martin's peptone solution to which have been added ground beef and dextrose is best suited for the preparation of the toxic culture filtrate.

(3) The blackleg toxin is susceptible to such influences as air, light, heat, etc., and in order to insure a potent product measures should be employed to minimize its exposure to the same.

(4) There is apparently a direct relation between the toxicity of the culture filtrate and its potency.

(5) Virulent bouillon cultures of the bacillus of symptomatic anthrax to which lactose has been added and which are then dried and pulverized give very satisfactory results as a test virus in standardization tests of blackleg immunizing agents on guinea pigs.

(6) There is apparently a distinct difference between the immunizing principles in blackleg natural aggressin and blackleg toxic culture filtrate.

(7) A uniformly satisfactory and practical method of isolating or concentrating the blackleg toxin is highly desirable.

CONCENTRATION OF SYMPTOMATIC ANTHRAX (BLACKLEG) TOXIN

[PRELIMINARY PAPER]

By WILLIAM N. BERG

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Following is a preliminary report on some experiments made for the purpose of devising a practical method for concentrating the toxin described by Kelser in the preceding paper. When received for concentration this toxin was a liquid obtained by filtering blackleg cultures through Berkefeld filters. Four cc. or more of this filtrate was a fatal dose for 350-gm. guinea pigs, killing within 48 hours when the dose was small(4–5 cc.) and in an hour if the dose was large (10 cc.). All of the toxicity tests in this report involve the intramuscular injection of the toxin or its concentrate into the leg of the experimental animal.

At first an attempt was made to precipitate the toxin with (1) alcohol, (2) half-saturation with ammonium sulphate, (3) saturation with ammonium sulphate, or (4) zinc chlorid. In general the chemical methods for the purification of tetanus toxin devised by Brieger and Boer [1] and by Hayashi [2] were followed, but without success. The precipitates obtained were not toxic to guinea pigs; the details of the chemical work will, therefore, be omitted. Foth [3] states (*p. 10, 21*) that the toxin can be precipitated out of a germ-free filtrate by an excess of absolute alcohol. Grassberger and Schattenfroh [4] in their monograph state that nontoxic products are obtained by drying the toxin *in vacuo* at 30° C. (*p. 21*) or by precipitation with ammonium sulphate or alcohol (*p. 24*).

The following method of drying the toxin to a paste which resembles the ordinary beef extract was successful on a laboratory scale. Experiments on large scale drying and on the keeping qualities of this product are under way. This method of drying has been applied by various investigators to the drying of meat, milk, and cultures. Among the first

[1] BRIEGER, L., and BOER. UEBER ANTITOXINE UND TOXINE. *In* Ztschr. Hyg. u. Infektionskrank., Bd. 21, Heft 2, p. 259–268. 1896.

——— ——— UEBER DIE TOXINE DER DIPTHERIE UND DES TETANUS. *In* Deut. Med. Wchnschr., Bd. 22, No. 49, p. 783–785. 1896.

[2] HAYASHI, H. WEITERE FORSCHUNGEN ÜBER DIE CHEMISCHE NATUR DES TETANUS-TOXINS. *In* Arch. Expt. Path. u. Pharmakol., Bd. 47, Heft 1/2, p. 9–18. 1901.

[3] FOTH, H. NEUE RAUSCHBRANDIMPFSTOFFE. *In* Ztschr. Infektionskrank. Haustiere, Bd. 10, Heft 1, p. 1–22. 1911.

[4] GRASSBERGER, R., and SCHATTENFROH, A. UEBER DAS RAUSCHBRANDGIFT UND EIN ANTITOXISCHES SERUM. 110 p. Leipzig und Wien, 1904.

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to use this method was Shackell,[1] who in 1909 (*p. 336*) pointed out the application of the method to the drying of a relatively unstable toxin.

Into each of several 9-, or 15-, cm. petri dishes, 10, or 25, cc. of the filtered toxin were transferred. These were kept overnight in a refrigerator at −9° C. (16° F.). The dishes containing the frozen toxin were then transferred to Hempel desiccators containing sulphuric acid, one large or three small dishes to one desiccator. The desiccators were evacuated with a Geryk pump to 2 to 3 mm. of mercury and then transferred to the refrigerator at −9° C., where they remained until the contents of the dishes had dried to a paste. This generally took from 24 to 48 hours. It is probable that the drying of this toxin must be accomplished while it is frozen; a few attempts at drying *in vacuo* at room temperature resulted in complete loss of toxicity. To some portions of the toxin, which was strongly alkaline, a calculated weight of acid potassium phosphate (KH_2PO_4) was added for the purpose of ascertaining the influence of neutralization of the alkali on the keeping qualities of the toxin paste.

Numerous inoculation tests were made on guinea pigs, using the dried toxin dissolved in water. The tests indicate that there was little, if any, loss in toxicity. The typical blackleg condition was found in animals that had died 24 hours or more after intramuscular injection of a weight of toxin paste corresponding to one fatal dose of the original toxin.

It was shown by Grassberger and Schattenfroh[2] that blackleg toxin can kill very quickly, without macroscopic lesions. In this respect it differs from tetanus and other toxins, which kill rather slowly, after producing noticeable pathological changes.

[1] SHACKELL, L. F. AN IMPROVED METHOD OF DESICCATION WITH SOME APPLICATIONS TO BIOLOGICAL PROBLEMS. *In* Amer. Jour. Physiol., v. 24, no. 3, p. 325-340. 1909.

[2] GRASSBERGER, R., and SCHATTENFROH, A. OP. CIT., p. 17.

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SOIL REACTION AND THE GROWTH OF AZOTOBACTER

[PRELIMINARY PAPER]

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INTRODUCTION

It has frequently been observed in this and other laboratories that, when soils are examined for Azotobacter, some give, on a mannite nutrient solution, a characteristic dark-brown film composed almost wholly of Azotobacter cells. Others give no visible surface growth. During the summer of 1917 a preliminary survey was conducted to ascertain to what extent soils in the vicinity of this Station exhibited the above variations. In all, 90 soils were collected within a radius of 2 miles of the laboratory. These samples were taken from as widely varying conditions as could be located. Some were collected from the highest hills and others from the lowest overflow bottom land, one even from a sand bar in the Kansas River. Samples of soil were taken from all of the following soil conditions: Cultivated, permanent alfalfa, pasture, roadsides, hedges, river and creek banks, and forests. Some of the spots from which samples were obtained were very fertile, while others were practically barren.

EXPERIMENTAL WORK

In collecting the soil for examination the ordinary precautionary methods used to prevent contamination were observed. The soil sample examined was a well-mixed composite of six or more smaller samples collected within a few yards of each other. When convenient, soil was taken to a depth of approximately 6 inches. As soon as possible the samples were brought to the laboratory, and sterile Erlenmeyer flasks containing 50 cc. of the following cultural solution were immediately inoculated. The composition of the cultural solution was: Di-potassium phosphate (K_2HPO_4), 0.2 gm., magnesium sulphate ($MgSO_4$), 0.2 gm., sodium chlorid (NaCl), 0.5 gm., mannite, 20 gm., ferric chlorid ($FeCl_3$), trace, distilled water, 1,000 gm. After all the salts had been dissolved, the solution was rendered slightly alkaline to phenolphthalein with sodium

hydroxid. Flasks were inoculated in quadruplicate with 10 cc. of a suspension made by shaking 100 gm. of soil in 200 cc. of sterile water. Two of the flasks were immediately sterilized, and all were incubated at room temperature for three weeks. The remaining soil was spread out in a thin layer, allowed to dry thoroughly, and stored for future physical and chemical study.

During incubation the growth was observed at frequent intervals, and microscopic examinations of the surface growth were made both at the end of one and at the end of three weeks. After incubation total-nitrogen determinations were made on all samples, and that present in the sterilized controls was deducted from that in the cultures. In all except a very few instances the growth in duplicate cultures was similar both macroscopically and microscopically. The quantity of nitrogen present in duplicates also checked within very narrow limits except in a few instances.

EXPERIMENTAL RESULTS

In Table I are given the sample number, date of collection, soil type, condition of ground, type of growth observed, average nitrogen fixed per culture, expressed in milligrams, and the reaction of the soil, expressed in P_H.

Under "Type of growth" the terms "typical," nontypical," and "none" have been used. Those designated as "typical" conform quite well with previously described cultures of *Azotobacter chroococcum*. The growth was a uniform brown to black film covering the entire surface and composed almost entirely of Azotobacter cells. The "nontypical" samples exhibited usually a heavy, more or less gelatinous, irregular, gray, yellowish, or even an irregularly brown spotted film. Under the microscope such a film was found to be composed of numerous types of bacteria, fungi, and protozoa. Always, however, there were large numbers of organisms similar to, if not identical with, Azotobacter. Those cultures designated as "none" gave very little, if any, surface growth and Azotobacter-like cells were never observed. In some instances such cultures exhibited a copious gas formation, while in others there was no visible evidence of growth. In most, if not all, cultures butyric acid was formed; especially was this true where gas formation took place.

In 37 samples, or 41 per cent, no Azotobacter developed. The nitrogen fixed in such cultures varied from −0.60 to 5.55 mgm. per culture, with an average of 3.88 mgm. In 28 samples, or 31 per cent, the growth was nontypical. The nitrogen fixed in these cultures varied from 3.41 to 9.95 mgm., with an average of 7.09 mgm. per culture. In 25 samples, or 28 per cent, the typical growth occurred. The quantity of nitrogen fixed in these cultures varied from 7.95 to 10.95 mgm., with an average per culture of 9.47 mgm.

TABLE I.—*Relation between soil type, condition of soil, growth of Azotobacter, nitrogen fixed per 50 cc. of culture, and reaction of soil solution*

Soil No.	Date.	Soil type.[a]	Condition of ground.	Type of growth.	Nitrogen-fixed.	Reaction expressed as P_H.
					Mgm.	
1	Apr. 19	Wabash silt loam	Cultivated	Typical	10.34	6.9
2	do	Colluvial Marshall silt loam	Forest	None	1.98	5.4
3	do	Marshall silt loam	Cultivated	do	2.98	5.6
4	do	Oswego silt loam	Sod	Nontypical	6.76	6.9
5	do	Marshall silt loam	Alfalfa	do	5.39	7.1
6	do	do	Cultivated	None	4.40	5.7
7	Apr. 23	do	Alfalfa	do	4.35	5.7
8	do	do	do	do	4.62	5.6
9	do	do	do	do	4.67	5.5
10	do	do	do	do	—.60	5.6
11	do	do	do	do	1.65	5.8
12	do	do	do	do	4.52	5.9
13	May 8	do	Cultivated	do	3.19	5.6
14	do	Oswego silt loam	Forest	Typical	8.52	7.4
15	do	do	do	do	10.28	7.4
16	do	Summit Silt loam	Cultivated	None	4.02	5.6
17	do	do	Pasture sod	do	3.02	5.5
18	do	Marshall silt clay loam	Cultivated	do	3.74	5.6
19	May 12	do	do	do	4.51	5.7
20	do	do	do	do	4.73	5.4
21	do	do	do	do	4.68	5.6
22	do	do	do	do	4.73	5.6
23	do	do	do	do	4.68	5.6
24	do	Oswego silt loam	Alfalfa	do	4.18	5.6
25	May 13	Wabash silt clay loam	Cultivated	Nontypical	6.38	7.0
26	do	do	Alfalfa	Typical	9.90	6.6
27	do	do	Cultivated	Nontypical	7.54	6.1
28	do	do	do	Typical	9.52	6.2
29	do	Marshall silt clay loam	Brook bottom	Nontypical	8.80	7.6
30	do	Wabash silt loam	Pasture sod	None	4.07	5.6
31	May 23	Laurel silt loam	Cultivated	Typical	10.62	7.5
32	do	Colluvial Summit silt loam	Alfalfa	None	5.55	5.9
33	do	do	Orchard	do	3.96	6.7
34	do	Summit silt loam	Alfalfa	do	4.51	6.8
35	do	Stony loam	Pasture sod	Nontypical	6.76	7.6
36	do	(?) Silt loam-	Cultivated	do	9.18	6.0
37	May 25	Wabash clay loam	do	None	5.44	6.2
38	do	Wabash silt clay loam	do	Nontypical	3.90	5.6
39	do	do	Orchard	do	8.58	6.1
40	do	Summit silt loam	Cultivated	do	8.14	7.0
41	do	Marshall silt loam	Alfalfa	do	7.59	6.0
42	do	Wabash silt loam	Cultivated	do	8.25	7.4
43	May 31	Laurel silt clay loam	do	Typical	9.62	7.7
44	do	Laurel very fine sandy loam	do	Nontypical	9.95	7.5
45	do	do	Orchard	Typical	10.12	7.4
46	do	Laurel fine sandy loam	Sod	None	1.04	5.9
47	do	Laurel very fine sandy loam	Hedge	Typical	10.01	7.4
48	do	do	Roadside	Nontypical	6.65	6.4
49	June 8	Marshall clay loam	Cultivated	None	2.75	5.5
50	do	Marshall silt loam	Alfalfa	do	3.96	5.8
51	do	do	Pasture sod	do	3.24	5.3
52	do	Summit silt loam	Forest	Nontypical	5.50	7.3
53	do	Marshall silt loam	do	Typical	9.02	7.7
54	do	Colluvial Summit silt loam	Orchard	Nontypical	7.42	6.0
55	June 12	do	Cultivated	Typical	8.69	7.5
56	do	Summit silt loam	Alfalfa	Nontypical	7.15	7.4
57	do	Oswego silt loam	Cultivated	do	7.59	7.5
58	do	Summit stony loam	Pasture sod	None	3.96	5.5
59	do	Summit silt loam	Cultivated	Typical	10.62	7.4
60	do	Oswego silt loam	Ravine	None	4.56	5.8
61	June 15	Wabash silt clay loam	Cultivated	do	3.68	5.5
62	do	(?) silt loam	Forest	Typical	7.26	7.5
63	do	Summit silt loam	Cultivated	do	10.95	7.4
64	do	do	do	do	8.30	6.1
65	do	Marshall silt loam	Orchard	None	5.17	5.7
66	do	do	Alfalfa	do	3.85	5.7
67	June 26	Wabash silt loam	Cultivated	do	4.07	5.5
68	do	Oswego silt loam	do	Nontypical	6.88	6.1
69	do	Marshall silt loam	do	None	4.12	5.6
70	do	do	Alfalfa	Typical	8.69	6.8
71	do	Wabash silt loam	Cultivated	Nontypical	6.87	5.6
72	do	Wabash silt clay loam	Pasture	do	6.76	7.0
73	June 28	Osage silt loam	Creek bank	None	4.51	5.9
74	do	do	Cultivated	Nontypical	8.08	7.4

[a] The writer is indebted to Prof. R. I. Throckmorton, of the Department of Agronomy, for the classification of the soils. Where the question mark (?) is used, it was impossible to identify the type with accuracy.

TABLE I.—*Relation between soil type, condition of soil, growth of Azotobacter, nitrogen fixed per 50 cc. of culture, and reaction of soil solution*—Continued

Soil No.	Date.	Soil type.	Condition of ground.	Type of growth.	Nitrogen fixed.	Reaction expressed as P_H.
					Mgm.	
75	June 28	Osage silt loam	Cultivated	Nontypical	6.08	7.5
76	..do	.....do	.....do	.....do	3.41	5.5
77	..do	.....do	Alfalfa	None	4.20	5.6
78	..do	Summit stony loam	Forest	Nontypical	5.50	7.7
79	June 30	Laurel medium sand	Sand bar	.....do	7.59	7.7
80	..do	Laurel very fine sandy loam	River bank	.....do	9.24	7.6
81	..do	Laurel fine sandy loam	Cultivated	Typical	9.62	7.4
82	..do	(?)	Forest	.....do	7.97	7.7
83	..do	Colluvial Summit silt loam	Cultivated	.....do	9.90	7.6
84	..do	Laurel fine sandy loam	Weedgrowth	.....do	9.51	7.5
85	July 11	Osage fine sandy loam	Cultivated	.....do	(a)	7.5
86	..do	Osage silt loam	.....do	Nontypical	(a)	7.5
87	..do	Summit stony loam	Creek bank	Typical	(a)	7.8
88	..do	Osage silt loam	Alfalfa	.....do	(a)	6.9
89	..do	Osage fine sandy loam	Creek bank	.....do	(a)	7.5
90	..do	Osage silt loam	Cultivated	.....do	(a)	7.3

a Quantitative nitrogen determination was not made.

DISCUSSION OF RESULTS

In order to show that the observed differences in Azotobacter growth and nitrogen fixation were not due to faulty technic, a soil known to possess a high nitrogen-fixing power was cultured in parallel as a control. In every instance this soil gave a typical film. The nitrogen fixed varied from 10.12 to 12.05 mgm., with an average of 10.50 mgm. per culture. There is evidently, therefore, a wide variation in the nitrogen-fixing power and in the Azotobacter development from the soils examined.

Efforts to correlate this variation with soil type, moisture content of soils, condition of soil with respect to cultivation, fertility, etc., gave negative results. In some instances soils of a similar type and collected very close to one another gave, on the one hand, good growth and nitrogen fixation, and, on the other, no surface growth. Many soils in high state of fertility gave no Azotobacter, while other almost barren or noncultivated soils gave excellent growth and high nitrogen fixation.

The only gross factor that the presence or absence of Azotobacter could in any way be associated with was the elevation from which the samples were taken. As a rule those soils coming from the higher elevations gave no Azotobacter growth, while those from the lower levels gave growth. There were, however, a number of marked exceptions to these rules. For example, soil 35 was from the top of a barren hill, while 37 was from low bottom land; No. 35 gave Azotobacter growth, while No. 37 did not.

The presence of Azotobacter in soils has frequently been associated both with the presence of calcium carbonate and with the reaction. From available evidence there seems to be no doubt that soils well supplied with calcium carbonate and necessarily alkaline give in cultural

solutions a more vigorous development of Azotobacter than do soils deficient in lime. There are, however, too many exceptions to this rule to regard the presence or absence of calcium carbonate alone as the controlling factor The available evidence regarding the influence of soil reaction upon the presence therein of Azotobacter has been obtained by methods which permit such wide discrepancy in results that they can be regarded only as indicative and not conclusive.

Christensen [1] has carried out by far the most carefully executed experiments along this line. In the work here referred to, 145 Danish soils from varying conditions were examined for Azotobacter, of which 53 per cent gave negative results. The reactions to litmus of 142 of these samples were recorded. The methods used are certainly not free from objections. Of 22 recorded as acid, only one gave Azotobacter. Fifty were recorded as neutral, and of these, 14 per cent gave Azotobacter. Eighty-seven per cent of the 70 recorded as alkaline gave positive cultural results.

The calcium-carbonate content of 136 of the same samples was determined by decomposing with concentrated hydrochloric acid and measuring the carbondioxid evolved. Forty-seven samples contained no calcium carbonate. Christensen states that all samples recorded as 0.05 per cent or less should be regarded as containing no carbonates. Of these 47, 32 per cent gave positive evidence of Azotobacter. Of 102 containing less than 0.10 per cent of calcium carbonate, 33 per cent gave Azotobacter cultures. There were 34 samples giving more than 0.10 per cent of calcium carbonate, and of these, 88 per cent gave Azotobacter, while all of the 23 samples containing more than 0.20 per cent gave positive results. None of the soils recorded as acid or neutral contained sufficient carbonates to replace the calcium carbonate of the cultural solution. Weis and Bornebusch,[2] however, studying the same problem in Danish forest soils found Azotobacter in only 2 out of 64 samples; nevertheless, 60 per cent of these soils contained sufficient carbonates to replace the calcium carbonate of Beijerinck's cultural solution. The last-named authors state that none of the soils examined by them could be regarded as requiring lime.

It would seem from the available experimental data that Azotobacter are capable of existing in many soils which contain none or only traces of calcium carbonate, and also in some soils reacting acid as ordinarily tested. The reaction, however, apparently plays a much more important rôle than the presence of calcium carbonate.

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[1] CHRISTENSEN, H. R. STUDIEN ÜBER DEN EINFLUSS DER BODENBESCHAFFENHEIT AUF DAS BAKTERIENLEBEN UND DEN STOFFUMSATZ IM ERDBODEN. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 43, No. 1/7, p. 1–166, 21 fig., 1 pl. Literatur, p. 163–165. 1915.

[2] WEIS, Fr., and BORNEBUSCH, C. H. OM AZOTOBACTERS FOREKOMST I DANSKE SKOVE, SAMT OM AZOTOBACTERFRØVENS BETYDNING FOR BESTEMMELSEN AF SKOVJORDERS KALKTRANG. (Abstract.) *In* Mo. Bul. Agr. Intel. and Plant Diseases, year 6, no. 4, p. 546–548. 1915. (Original article in Forstl. Forsøgsvaesen Danmark, Bd. 4, Haefte 4, p. 319–337. 1914. Not seen.)

Very few, if any, local soils are regarded, agriculturally speaking, as deficient in lime. In fact, the application of lime far in excess of supposed requirements to soils on the Agronomy farm of the Kansas Experiment Station has been without effect upon productivity even when alfalfa was grown. Many of the soils herein reported as containing no Azotobacter were collected from the Agronomy farm. Sample 12 is from a plat to which lime has been applied, yet which failed to show Azotobacter.

Since the writer was unable to associate the presence of Azotobacter with any other factor studied, and since the apparent correlation of their presence with soil reaction was known, this factor was deemed worthy of investigation. A study of the influence of soil reaction seemed especially important, since more exact methods are now available for determining soil acidity.

Recent investigation in other lines of bacteriology have shown that in many instances the degree of acidity or hydrogen-ion concentration is perhaps much more important in controlling bacterial activity than the total or titratable acidity. It has been shown in a number of instances that the degree of acidity tolerated by certain species of bacteria has a very definite limit. Furthermore, none of the methods in vogue for ascertaining the total or titratable acidity of soils are very satisfactory. For these reasons it was thought best, if possible, to determine the reaction in terms of hydrogen-ion concentration. For this purpose the writer has made use of the colorimetric method outlined by Clark and Lubs[1] as recently modified for soils by Gillespie.[2] The indicators used were methyl red, bromcresol purple, bromthymol blue, and phenol red. The standard hydrogen-ion-concentration solutions were prepared as directed by Clark and Lubs, and their accuracy tested and corrected, if need be, by means of electrometric measurements. Little difficulty was experienced in checking with different indicators except in those solutions falling on the acid side of bromcresol purple and the alkaline side of methyl red. Perhaps propyl red would have obviated this difficulty, but none was available when these analyses were made. However, any error arising from this difficulty can in no way vitiate conclusions that may be drawn from these experiments.

In the last column of Table I is given the hydrogen-ion concentration observed in the soil extract, expressed in the usual way—that is, P_H. These results are certainly very striking. Of those soils in which no Azotobacter were observed, all with the exception of three gave a P_H of 5.9 or less. All of the soils which gave Azotobacter growth,

[1] CLARK, W. M., and LUBS, H. A. THE COLORIMETRIC DETERMINATION OF HYDROGEN ION CONCENTRATION AND ITS APPLICATIONS IN BACTERIOLOGY. *In* Jour. Bact., v. 2, no. 1, p. 1–34; no. 2, p. 109–136, no. 3, p. 191–236, 8 fig. 1917. References, p. 233–236.

[2] GILLESPIE, L. J. THE REACTION OF SOIL AND MEASUREMENTS OF HYDROGEN-ION CONCENTRATION. *In* Jour. Wash. Acad. Sci., v. 6, no. 1, p. 7–16, 2 fig. 1916.

except three, gave a P_H of 6.0 or above. The average P_H of soils showing no Azotobacter growth was 5.71 and the nitrogen fixed 3.88 mgm. per culture. The average P_H of those soils showing Azotobacter growth was 6.78 and the average nitrogen fixed was 8.11 mgm. per culture. Of the exceptions to the above rule, soil 38 gave very few isolated colonies, and in the case of No. 76 only one culture gave Azotobacter. In these two instances the Azotobacter growth was probably due to contamination. All these exceptions are being studied further.

It should be remembered that the acidity analyses were made on samples of soil that had been stored from 7 to 10 months. Gillespie has called attention to the possibility of determinations made under such conditions varying slightly from actual soil conditions.

The writer does not, therefore, wish to leave the impression that the the maximum acidity tolerated by Azotobacter is necessarily represented exactly by a P_H of 6.0, or that the limits are necessarily so definite as these experiments would indicate. It is believed, however, that the results herein reported are very significant. Also that when investigations now under way are completed, the writer will be in a position to say that active Azotobacter will not exist in soils in which, other factors not interfering, the hydrogen-ion concentration exceeds a fairly definite limit. He hopes also to give much more accurate data as to what that limit is. The same phenomenaare being studied in the case of other soil organisms.

EFFECT OF DIFFERENT OXYGEN PRESSURES ON THE CARBOHYDRATE METABOLISM OF THE SWEET POTATO

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INTRODUCTION

The recognizable products which are formed as a result of starch transformation in the sweet potato (*Ipomoea batatas*) during storage are reducing sugars and cane sugar. According to Miyaki [1] the reducing sugars consist of glucose and possibly fructose. Maltose has not been found. The main product is cane sugar, which has been frequently identified.

In the course of ordinary storage the monosaccharids soon reach their maximum concentration, which in Big Stem and Southern Queen rarely exceeds 2 per cent of the weight of the fresh potato. The cane sugar continues to accumulate until in the varieties named it represents as much as 7 per cent of the fresh potato.[2]

The fact that the reducing sugars remain at a low concentration while the cane sugar continues to accumulate suggested that the reducing sugar is an intermediate product in the transformation of starch to cane sugar in the sweet potato in storage. Evidence that the changes proceed in this manner was obtained by a study of the process at low temperatures by which the rates of the different steps in the series of changes are unequally modified.[3] It was thus shown that the production of reducing sugar antecedes the formation of cane sugar. That a further separation of the various steps in this transformation, or possibly a suppression of one or more of the phases of the process, could be brought about by other means, such as changes in oxygen pressure, seemed not improbable, especially since Cruickshank in 1797[4] had observed that soaked barley seeds failed to become sweet in the absence of oxygen, and Boysen-Jensen [5] more recently found that cane sugar was not formed

[1] MIYAKI, K. ON THE NATURE OF THE SUGARS FOUND IN THE TUBERS OF SWEET POTATOES. *In* Jour. Biol. Chem., v. 21, no. 2, p. 503–506. 1915.

[2] HASSELBRING, Heinrich, and HAWKINS, L. A. PHYSIOLOGICAL CHANGES IN SWEET POTATOES DURING STORAGE. *In* Jour. Agr. Research, v. 3, no. 4, p. 331–342. 1915. Literature cited, p. 341–342.

[3] HASSELBRING, Heinrich, and HAWKINS, L. A. CARBOHYDRATE TRANSFORMATIONS IN SWEET POTATOES. *In* Jour. Agr. Research, v. 5, no. 13, p. 543–560. 1915.

[4] CRUICKSHANK, William. SOME EXPERIMENTS AND OBSERVATIONS ON THE NATURE OF SUGAR. *In* Rollo, John. An account of two cases of the diabetes mellitus . . . v. 2, p. 210–226. London, 1797. Reprinted in Jour. Nat. Phil., Chem., and Arts [Nicholson], v. 1, p. 337–341. 1797. French trans. by Guyton in Ann. Chim., v. 25, p. 37–50. 1798.

[5] BOYSEN-JENSEN, P. ÜBER SYNTHETISCHE VORGÄNGE IM PFLANZLICHEN ORGANISMUS. I. DIE ROHRZUCKERSYNTHESE. *In* Biochem. Ztschr., Bd. 40, Heft 5/6, p. 420–440, 2 fig. 1912.

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in germinating barley and peas under similar conditions. With this idea in view a study of the effects of different oxygen pressures on the carbohydrate transformation in the sweet potato was undertaken.

EXPERIMENTAL METHODS

The general method adopted was that heretofore used of cutting sweet potatoes into halves lengthwise and storing one half under experimental conditions, while the other half was prepared for immediate analysis. The potatoes were always dug in the afternoon, properly cleaned, and stored in a cool place until the following day, when they were prepared for the experiments. Either five or six individuals were used for each experiment.

With respect to the total gas pressure to which the stored halves were subjected, the experiments may be divided into three groups: (1) experiments at pressures greater than one atmosphere; (2) experiments at atmospheric pressure; and (3) one experiment at a pressure of less than one atmosphere.

In the experiments at pressures greater than one atmosphere the potatoes were stored in a gas-tight iron cylinder 33 cm. high and 22.9 cm. in diameter. The pressures were measured by means of a standard test gauge in the head of the cylinder. The whole apparatus, except the projecting gauge, was submerged in a constant temperature water bath. Preliminary experiments showed that with an inside pressure of 10 atmospheres there was no leakage from the cylinder during a period of 10 days.

In the second group of experiments the potatoes were stored in oxygen, air, or hydrogen at atmospheric pressure. For this purpose desiccators kept in constant temperature chambers were used. For the experiments in air the desiccators were merely ventilated, but in the other experiments the gases were passed through the desiccators in a rapid stream until all the air had been replaced. Thereupon the current was slowed down until 60 to 100 bubbles of gas per minute passed through wash bottles at the exits. Before entering the desiccators the gases passed through about 10 meters of copper tubing coiled in the constant temperature chambers and then through wash bottles filled with water. Short thermometers placed in these wash bottles showed that the gases entered the desiccators at the temperature of the chambers. The oxygen and hydrogen were obtained from cylinders furnished by a commercial company. The hydrogen contained approximately 0.17 to 0.25 per cent of oxygen. In two experiments, as will be mentioned later, these small traces of oxygen were removed from the hydrogen.

The single experiment in which the potatoes were stored at less than atmospheric pressure was carried out by means of the gas cylinder described above.

EXPERIMENTAL WORK

EXPERIMENTS AT PRESSURES GREATER THAN ONE ATMOSPHERE

Two experiments were conducted under pressures greater than one atmosphere. These require only a brief discussion, since in both cases the sweet potatoes were killed. In the first experiment the potatoes were stored for 10 days in air at 30° C. and under a pressure of 10 atmospheres. At the end of that period the potatoes showed a few spots where organisms had developed; otherwise the tissues were intact but killed. No analyses of these were made. In the second experiment the potatoes were kept for 5 days in oxygen at 30° and at a pressure of 5 atmospheres. These also were killed. The tissues were watery but firm and, so far as could be observed microscopically, were free from fungi and bacteria. Two of the halves were analyzed. The results are given in Table I. In this and in the subsequent tables the halves analyzed at the beginning of the experiment are marked "a," and the stored halves are marked "b."

TABLE I.—*Changes in composition of sweet potatoes stored for 5 days in oxygen at 30° C. and at a pressure of 5 atmospheres*

Sweet potato No.	On the basis of fresh material.				On the basis of dry matter.		
	Moisture.	Starch.	Reducing sugar as glucose.	Cane sugar.	Starch.	Reducing sugar as glucose.	Cane sugar.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1a	75.50	17.09	0.31	1.63	69.76	1.27	6.65
1b	73.85	17.78	1.75	.63	67.99	6.69	2.41
6a	71.14	21.39	.40	1.72	74.12	1.39	5.96
6b	71.04	20.70	1.81	.95	71.48	6.25	3.28

The data show that under these conditions the hydrolysis of starch proceeded to a very limited extent, and that not only did no synthesis of cane sugar take place but cane sugar disappeared. As a result of the hydrolysis of starch and cane sugar there was a considerable accumulation of reducing sugar. To what extent these changes took place in the living potato it is not possible to say. It is likely from the disappearance of cane sugar that the roots were quickly killed and that the synthesis of cane sugar at least does not go on in killed tissues. The hydrolysis of starch also is greatly retarded. Since the whole problem of the effects of high pressures on the metabolism of the plant organs requires a detailed investigation, no further experiments in this field were conducted at this time.

EXPERIMENTS AT ATMOSPHERIC PRESSURE

In the first set of experiments at atmospheric pressure different lots of halved sweet potatoes were stored for five days in oxygen, air, and

hydrogen, respectively, at a temperature of 30° C. The changes in composition of the stored halves as compared with the halves analyzed immediately are given in Table II.

TABLE II.—*Changes in composition of sweet potatoes stored for five days under different conditions*

Sweet potato No.	On the basis of fresh material.				On the basis of dry matter.		
	Moisture.	Starch.	Reducing sugar as glucose.	Cane sugar.	Starch.	Reducing sugar as glucose.	Cane sugar.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
STORED IN OXYGEN AT 30° C. AND AT ATMOSPHERIC PRESSURE							
7a	77.07	15.16	0.21	1.82	66.11	0.92	7.94
7b	76.96	11.92	1.10	3.97	51.74	4.77	17.23
8a	75.96	15.87	.23	2.10	66.02	.96	8.74
8b	76.65	13.23	.98	3.27	56.66	4.20	14.00
9a	76.10	15.80	.30	2.08	66.12	1.26	8.70
9b	74.87	13.74	1.34	3.81	54.68	5.33	15.16
10a	76.70	15.75	.43	1.94	67.60	1.84	8.33
10b	77.08	12.57	1.69	2.98	54.84	7.37	13.00
11a	76.01	16.19	.25	1.95	67.49	1.04	8.13
11b	73.72	15.07	1.23	3.35	57.34	4.68	12.75
12a	76.32	15.79	.32	2.13	66.68	1.35	9.00
12b	77.65	11.89	1.39	3.52	53.20	6.22	15.75
STORED IN AIR AT 30° C. AND AT ATMOSPHERIC PRESSURE							
13a	76.28	15.92	0.29	2.09	67.12	1.22	8.81
13b	76.35	14.05	1.45	3.21	59.41	6.13	13.57
14a	75.78	16.18	.30	2.19	66.81	1.24	9.04
14b	75.87	14.97	1.32	2.10	62.04	5.47	8.70
15a	77.54	14.69	.25	2.11	65.40	1.11	9.39
15b	77.04	13.70	1.15	2.67	59.67	5.01	11.63
16a	76.46	16.06	.41	1.75	68.22	1.74	7.43
16b	75.90	15.03	1.46	2.31	62.37	6.06	9.59
17a	75.02	16.80	.25	2.11	67.26	1.00	8.45
17b	74.95	15.18	.77	3.11	60.60	3.07	12.42
18a	76.08	15.78	.25	2.12	65.97	1.05	8.86
18b	74.75	15.03	.85	3.16	59.53	3.37	12.52
STORED IN HYDROGEN AT 30° C. AND AT ATMOSPHERIC PRESSURE							
19a	76.47	15.74	0.18	1.78	66.89	0.77	7.56
19b	77.26	11.94	29	4.36	52.51	1.28	19.17
20a	77.15	15.57	23	1.84	68.14	1.01	8.05
20b	77.47	12.44	.28	4.15	55.22	1.24	18.42
21a	78.08	14.63	.17	1.93	66.75	.76	8.80
21b	78.18	11.75	.34	4.50	53.85	1.56	20.62
22a	76.83	15.11	.16	2.30	65.21	.69	9.93
22b	77.50	11.69	.26	4.78	51.96	1.16	21.25
23a	76.86	15.63	.18	2.03	67.55	.78	8.77
23b	77.16	12.57	.32	4.50	55.04	1.40	19.70
24a	77.60	15.30	.41	1.61	68.30	1.83	7.19
24b	79.01	10.93	.41	4.22	52.07	1.95	20.10

At the end of the experiment the stored halves were fresh and crisp and in perfect condition. The only difference noted between those stored in air or oxygen and those stored in hydrogen was that in the roots stored in air or oxygen the oxidizable chromogenic material exuding from the cut surfaces was darkened, while in those stored in hydrogen discoloration was entirely absent, but appeared as soon as the potatoes were exposed to the air.

These data show that the formation of cane sugar[1] in the sweet potato is not inhibited nor depressed in an atmosphere practically free from oxygen. In regard to the quantitative effects of the different oxygen pressures it seems clear that both in an atmosphere of oxygen and in an atmosphere practically free from oxygen more starch disappears and more cane sugar is formed than in air. The percentage of cane sugar is greatest in the potatoes stored in hydrogen.

Since cane sugar is apparently not utilized in respiration by the sweet potato, the loss of other materials through respiration would result in an increase of the percentage of cane sugar without an increase in the actual quantity present. However, the possible difference in loss of material by respiration in air and in hydrogen is not sufficiently great to account for the greater percentage of cane sugar in the potatoes stored in hydrogen. It appears, therefore, that in the absence of oxygen cane sugar is actually produced more rapidly than in air. A further fact worthy of note is that at the temperature of these experiments there is practically no increase in reducing sugar in the potatoes stored in hydrogen.

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[1] That the nonreducing sugar formed in the sweet potato in the absence of oxygen is cane sugar was shown by the following experiments:

Four small sweet potatoes, weighing together 1075.5 gm., were cut into halves. One lot of halves weighing 555.5 gm. was grated immediately. From the mash three 25-gm. samples were taken for sugar determinations. The remaining mash, after the removal of these samples, weighed 475 gm., some loss having resulted from the adherence of material to the grater. From this mash the sugar was quantitatively extracted with 70 per cent alcohol, first by repeated decantation in the cold, and finally by means of a Soxhlet apparatus. After concentration of the extract under reduced pressure the sugar was isolated as barium saccharate, from which it was recovered in crystallized form. The quantity of nonreducing sugar present in the mash, according to determination, was 14.9 gm. The sugar recovered from the barium saccharate, after having been washed with glacial acetic acid and alcohol, weighed 13.22 gm.; 0.6014 gm. dissolved and made up to 50 cc. at 20°C., gave an angular rotation in a 4-dcm. tube of 3.169°, to the right equivalent to a specific rotation of +65.9°. The remainder of the sugar was recrystallized from alcohol and yielded 12.05 gm.; 0.7440 gm. dissolved and made up to 50 cc. as before gave an angular rotation of 3.953°, or a specific rotation of +66.4°.

The second lot of halves, weighing 519.5 gm., was stored for 15 days in the manner described in the text in an atmosphere of hydrogen entirely freed from oxygen. At the end of that period these halves, whose weight had decreased to 505.0 gm., were treated like the first lot. The mash, after removal of the samples for sugar determination weighed, 421 gm., and according to determination contained 24.42 gm. of nonreducing sugar. The quantity theoretically present in the equivalent of the mash

before the experiment was 13.60 gm., correction having been made for the loss of weight of the halves during the experiment. The yield from the first crystallization was 22.47 gm., 0.7357 gm. dissolved and made up to 50 cc. at 20°C. gave an angular rotation in a 4-dcm. tube of 3.886° to the right, or a specific rotation of +66°. The residue on recrystallization yielded 20.69 gm.; 0.5450 gm. dissolved as before gave an angular rotation of 2.896°, equivalent to a specific rotation of +66.4°. The specific rotation of cane sugar is 66.5°. The quantity of sugar recovered from the last recrystallization is 7.09 gm. in excess of the quantity present in an equivalent of the mash before the experiment.

In a second experiment a single large sweet potato weighing 884.5 gm. was used. The potato was cut lengthwise and the halves treated as described above, with the exception that the mash was extracted first by decantation and finally by percolation in the cold. The stored half, weighing 471 gm., lost 12 gm. during the experiment. From the mash of the first half, 9.25 gm. of recrystallized sugar were obtained; 4.4860 gm. of this dissolved and made up to 50 cc at 20° C. gave an angular rotation in a 2 dcm. tube of 11.972°, or a specific rotation of +66.7°. The yield of recrystallized sugar from the stored half was 19.02 gm.; 4.1003 gm. dissolved as before gave an angular rotation of 10.892°, or a specific rotation of +66.4°. The quantity of nonreducing sugar originally present in the equivalent of this mash, according to determination and after correction for the loss of weight during storage, was 13.31 gm. The recrystallized sugar therefore represents an increase of 5.71 gm.

The complete data from these experiments are tabulated here.

	First experiment.		Second experiment.	
	I.	II. (Stored half).	I.	II. (Stored half).
Weight of halves....gm..	555.5	519.5	413	471
Weight of stored half at end of experiment....gm..	....	505	....	459
Weight of mash extracted....gm..	475	421	332	376
Weight of sucrose present according to determination....gm..	14.91	24.42	11.45	22.37
Sucrose in mash of II at beginning of experiment....gm..	....	13.60	....	13.31
Yield of sugar from first crystallization.gm..	13.22	22.47	10.67	21.50
Specific rotation at 20°C....°..	+65.9°	+66.0°	....	....
Yield of sugar after one recrystallization.gm..	12.05	20.69	9.25	19.02
Specific rotation at 20°C....°..	+66.4	+66.4	+66.7	+66.4

The specific rotation of the recrystallized product obtained in these experiments identifies as cane sugar the non-reducing sugar present in the freshly dug sweet potato as well as the additional non-reducing sugar formed during storage in the absence of oxygen. To Dr. C. S. Hudson, of the Bureau of Chemistry, the writer is indebted for helpful suggestions regarding the isolation and identification of the sugar from the sweet potato.

A second series of experiments was carried out under the same conditions but the halves were kept in the desiccators for 10 days. At the end of that period the potatoes were in perfect condition, as in the first experiments. The results of this series are given in Table III.

The statements made in regard to the first set of experiments apply equally well to this set. In general the change in the 10-day period is scarcely greater than that during the 5-day period. Only in the potatoes

stored in air is a noticeable further increase of sugar apparent. In oxygen and in hydrogen a state of equilibrium is practically reached in five days. In these experiments also there is only a slight increase in reducing sugar in the absence of oxygen.

TABLE III.—*Changes in composition of sweet potatoes stored for 10 days under different conditions*

Sweet potato No.	On the basis of fresh material.				On the basis of dry matter.		
	Moisture.	Starch.	Reducing sugar as glucose.	Cane sugar.	Starch.	Reducing sugar as glucose.	Cane sugar.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
STORED IN OXYGEN AT 30° C. AND AT ATMOSPHERIC PRESSURE							
25a	77.31	15.31	0.31	1.92	67.48	1.37	8.46
25b	76.66	13.57	1.68	2.68	58.14	7.20	11.48
26a	75.94	16.45	.22	1.71	68.37	.91	7.11
26b	76.88	12.81	1.20	3.32	55.41	5.19	14.36
27a	75.77	16.63	.32	2.12	68.64	1.32	8.75
27b	76.14	13.77	1.44	3.21	57.71	6.04	13.46
28a	73.85	18.76	.23	1.94	71.74	.88	7.42
28b	75.07	14.63	1.33	3.64	58.69	5.34	14.60
29a	72.82	19.81	.42	1.90	72.88	1.55	6.99
29b	72.40	17.37	1.37	3.09	62.94	4.96	11.20
30a	71.94	20.70	.34	1.93	73.77	1.21	6.88
30b	71.96	17.42	1.42	3.40	62.13	5.06	12.13
STORED IN AIR AT 30° C. AND AT ATMOSPHERIC PRESSURE							
31a	75.86	15.90	0.30	2.55	65.87	1.24	10.56
31b	74.63	15.58	1.36	3.16	61.41	5.37	12.46
32a	73.47	18.41	.23	2.38	69.39	.87	8.71
32b	72.72	17.19	.79	3.45	63.01	2.89	12.65
33a	73.97	18.04	.27	2.40	69.31	1.04	9.22
33b	73.97	15.96	.83	3.57	61.32	3.19	13.72
34a	76.14	15.97	.24	2.13	66.93	1.00	8.93
34b	75.40	14.49	1.06	3.56	58.90	4.31	14.47
35a	76.33	16.24	.45	2.05	68.61	1.90	8.66
35b	75.26	14.50	1.17	3.63	58.61	4.73	14.67
36a	74.59	17.55	.36	2.48	69.07	1.42	9.76
36b	73.80	15.93	1.07	3.70	60.80	4.08	14.12
STORED IN HYDROGEN AT 30° C. AND AT ATMOSPHERIC PRESSURE							
37a	75.10	17.42	0.27	2.00	69.96	1.08	8.03
37b	77.21	12.56	.51	4.26	55.11	2.24	18.69
38a	75.73	16.21	.18	2.43	66.79	.74	10.01
38b	77.07	12.27	.28	4.51	53.51	1.22	19.67
39a	74.70	17.33	.17	2.22	68.50	.67	8.77
39b	75.89	13.34	.31	4.48	55.33	1.29	18.58
40a	74.55	17.59	.22	2.37	69.12	.86	9.31
40b	76.24	13.50	.29	4.39	56.82	1.22	18.48
41a	75.80	16.26	.28	2.36	67.19	1.16	9.75
41b	75.84	13.75	.27	4.64	56.91	1.12	19.21
42a	74.50	18.21	.27	1.87	71.41	1.06	7.33
42b	75.64	14.43	.37	4.03	59.24	1.52	16.54

In experiments reported in previous papers it was found that an increase in reducing sugar precedes or accompanies the increase in cane sugar in the sweet potato. From these observations the conclusion was drawn that the monosaccharides result from the hydrolysis of starch, and that cane sugar is synthesized from these. The failure of reducing sugar to accumulate in an atmosphere containing only traces of oxygen might be attributed to two causes. First, to its more rapid utilization in the formation of cane sugar, and, second, to a greater demand for materials to sustain anaerobic respiration at the temperature at which the experiments were conducted. If, therefore, these processes, especially the respiration, could be retarded without retarding in a corresponding degree the hydrolysis of starch reducing sugar ought to accumulate in the absence of oxygen in the same manner as in air. To settle this point, a series of four experiments was carried out in which the potatoes were stored for different lengths of time from 3 to 20 days in hydrogen at a temperature of 4.5° C.

Since, as has been stated, the hydrogen used in these experiments contained traces of oxygen and as no tendency toward the suppression of the formation of cane sugar was evident, it might be urged that the small traces of oxygen mixed with the hydrogen from the cylinders were sufficient to stimulate the processes leading to the formation of cane sugar. It therefore became necessary to exclude these traces in order to determine definitely whether cane sugar could be formed in the sweet potato in the absence of all traces of oxygen. For this purpose the hydrogen used in the last two experiments (Table IV, 10 and 20 days) was passed through a tube containing heated palladium asbestos. Analyses over mercury of the gas issuing from the chambers made on the day after the experiments were set up showed no oxygen present.

The results of this series of experiments are given in Table IV.

Table IV shows clearly the course of the carbohydrate changes in the absence of oxygen. After three days almost no change has taken place. After five days an increase in reducing sugar becomes apparent, but the increase in cane sugar is very slight. At the end of 10 days the reducing sugar has increased to from two to four times the original quantity, while the cane sugar still shows but very little increase. During the next 10 days there is a further increase in reducing sugar, but this period is characterized mostly by the great increase in cane sugar.

These facts show that the carbohydrate transformations in the sweet potato proceed in the same manner under anaerobic conditions as they do under aerobic conditions. The failure of reducing sugar to accumulate at high temperatures under anaerobic conditions is probably in part attributable to its more extensive utilization in respiration.

The data in the last two experiments (Table IV, 10 and 20 days) show that even in the entire absence of oxygen the formation of cane sugar is possible in the sweet potato. It is evident, therefore, that no significance need be attributed to the effects of the small traces of oxygen contained in the hydrogen used in the other experiments.

TABLE IV.—*Changes in composition of sweet potatoes stored in hydrogen at 4.5° C. and at atmospheric pressure for different periods*

Sweet potato No.	On the basis of fresh material.				On the basis of dry matter.		
	Moisture.	Starch.	Reducing sugar as glucose.	Cane sugar.	Starch.	Reducing sugar as glucose.	Cane sugar.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
STORED FOR 3 DAYS							
43a	73.38	19.00	0.32	1.96	71.37	1.20	7.36
43b	73.72	18.81	.43	2.15	71.58	1.64	8.18
44a	75.76	16.90	.43	2.11	69.72	1.77	8.70
44b	75.15	17.30	.53	2.16	69.62	2.13	8.69
45a	73.70	18.88	.30	2.04	71.79	1.14	7.76
45b	72.07	20.27	.23	2.18	72.57	.82	7.81
46a	73.56	19.04	.47	2.16	72.01	1.78	8.17
46b	73.74	18.77	.65	2.34	71.48	2.48	8.91
47a	73.21	19.31	.35	2.05	72.08	1.31	7.65
47b	72.96	19.49	.41	2.10	72.08	1.52	7.77
STORED FOR 5 DAYS							
48a	73.34	19.07	0.35	2.30	71.53	1.31	8.63
48b	75.10	16.93	.66	2.31	67.99	2.65	9.28
49a	72.35	20.18	.24	1.97	72.98	.87	7.12
49b	73.10	18.88	.47	2.21	70.19	1.75	8.22
50a	73.99	18.79	.31	1.77	72.24	1.19	6.81
50b	73.65	18.50	.58	1.94	70.21	2.20	7.36
51a	71.44	21.28	.38	1.92	74.51	1.33	6.72
51b	73.27	18.70	.89	2.08	69.96	3.33	7.78
52a	75.09	17.61	.35	2.07	70.70	1.41	8.31
52b	74.30	17.76	.56	2.21	69.11	2.18	8.60
STORED FOR 10 DAYS							
53a	74.58	17.55	0.38	2.07	69.04	1.49	8.14
53b	76.16	15.73	1.48	1.92	65.98	6.21	8.05
54a	74.76	17.78	.41	2.34	70.44	1.62	9.27
54b	75.30	16.47	1.39	2.29	66.68	5.63	9.27
55a	73.28	18.99	.45	2.66	71.07	1.68	9.96
55b	74.06	17.66	1.08	2.94	68.08	4.16	11.33
56a	71.07	21.58	.42	2.23	74.60	1.45	7.71
56b	72.15	19.96	1.03	2.59	71.67	3.70	9.30
57a	75.64	16.90	.58	2.18	69.38	2.38	8.95
57b	76.73	15.05	1.69	1.94	64.68	7.26	8.34
STORED FOR 20 DAYS							
58a	76.34	16.52	0.59	1.98	69.82	2.49	8.37
58b	77.60	13.22	1.51	2.87	59.02	6.74	12.81
59a	76.37	16.43	.82	2.02	69.53	3.47	8.55
59b	77.88	12.93	1.76	2.91	58.46	7.96	13.15
60a	76.57	16.40	.70	1.80	70.00	2.99	7.68
60b	78.34	12.94	1.40	2.92	59.74	6.46	13.48
61a	76.52	16.17	.59	1.89	68.87	2.51	8.05
61b	77.05	13.32	1.43	3.30	58.04	6.23	14.38
62a	75.68	16.78	.63	2.17	69.00	2.59	8.92
62b	77.56	12.79	1.67	3.29	57.00	7.44	14.66

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EXPERIMENT UNDER A PRESSURE OF LESS THAN ONE ATMOSPHERE

A single experiment was carried out in which the sweet potatoes were stored in a vacuum chamber which was kept in a water bath at 30° C. For this purpose the gas cylinder described above was used. The air was exhausted to a pressure of 4 mm. A dish of moist soda-lime was placed on the bottom of the chamber to absorb the carbon dioxid. The potatoes were kept in the chamber for five days. Owing to the absorption of water by the soda-lime the interior of the chamber was very dry and the potatoes were much wilted, but otherwise uninjured. The results of this experiment are given in Table V.

TABLE V.—*Changes in composition of sweet potatoes stored for 5 days at 30° C. and at a pressure of 4 mm.*

Sweet potato No.	On the basis of fresh material.				On the basis of dry matter.		
	Moisture.	Starch.	Reducing sugar as glucose.	Cane sugar.	Starch.	Reducing sugar as glucose.	Cane sugar.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
63a	76.74	15.51	0.35	2.27	66.68	1.54	9.76
63b	76.78	10.29	.55	6.06	44.32	2.37	26.10
64a	76.88	15.27	.32	2.30	66.05	1.38	9.95
64b	76.80	10.61	.47	5.87	45.73	2.03	25.30
65a	78.19	13.97	.50	2.22	64.05	2.29	10.18
65b	76.29	10.18	.91	6.11	42.94	3.84	25.77
66a	77.59	14.59	.29	2.45	65.10	1.29	10.93
66b	70.87	14.83	.31	6.45	50.91	1.06	22.14
67a	75.01	16.87	.24	2.29	67.51	.96	9.16
67b	73.86	12.09	.38	6.44	46.25	1.45	24.64
68a	77.66	14.40	.43	2.33	64.46	1.92	10.43
68b	77.94	10.15	.61	5.43	46.01	2.77	24.61

It is very probable that the available oxygen within the cylinder was soon removed by the potatoes and that thereafter they were in an atmosphere free from oxygen. The behavior of the potatoes under these conditions is like that of the potatoes stored in hydrogen at the same temperature. There is a marked accumulation of cane sugar but scarcely any increase in reducing sugar.

DISCUSSION OF RESULTS

In the experiments of Cruickshank the lack of sweet taste in the soaked barley kept in an atmosphere free from oxygen may be taken to indicate the absence not only of cane sugar, which, according to O'Sullivan,[1] may constitute as much as 4.5 per cent of the dry weight of the germinated grain, but also of the other sugars occurring in malt. It is therefore reasonably sure that under the conditions of the experiments no cane sugar was formed. In like manner the experiments of Boysen-Jensen show that in

[1] O'SULLIVAN, C. ON THE SUGARS OF SOME OF THE CEREALS AND OF GERMINATED GRAIN. (Abstract.) *In* Chem. News, v. 52, no. 1359, p. 293. 1885.

the absence of oxygen cane sugar is not formed in germinating peas and barley. Both of these investigators conclude that the presence of oxygen is one of the necessary conditions for the formation of cane sugar. This conclusion, however, is not of general validity, since the experiments with sweet potatoes show that cane sugar can be formed in some plant organs in the absence of oxygen. Possibly this difference in behavior of sweet potato roots and germinating seeds is associated with the difference in the degree of activity between dormant organs and active embryos or seedlings.

From the correlation which he observed between the formation of cane sugar and aerobic respiration Boysen-Jensen believes that the respiratory process furnishes the energy necessary for the synthesis of cane sugar. If such a relation between respiratory energy and sugar synthesis exists it is not surprising that in some cases, as in the sweet potato, the requisite energy can be derived also from the processes of anaerobic respiration. In such cases, however, we should expect to find the quantity of material consumed in anaerobic respiration greater than that consumed in normal respiration, since the energy derived from a given mass of material is not equal in the two cases.

Two lots of halved sweet potatoes weighing, respectively, 1,273 and 1,479 gm., were placed in respiration chambers at 30° C. Through the first chamber a rapid current of air was passed for three days and through the second a current of hydrogen was passed in the same manner. The daily carbon-dioxid output in grams per kilogram of the two lots of roots for the next five days was as follows:

LOT I: POTATOES IN AIR.	LOT II: POTATOES IN HYDROGEN.
1.44	1.67
1.32	1.81
1.38	1.85
1.12	2.03
1.01	2.32

On the hypothesis that in normal respiration glucose is completely oxidized to carbon dioxid and water, while in anaerobic respiration carbon dioxid and alcohol are formed, 1 gm. of carbon dioxid in normal respiration is equivalent to 0.682 gm. of glucose and in anaerobic respiration to 2.045 gm. of glucose. It is seen, therefore, that the quantity of material consumed in anaerobic respiration is actually much greater than that consumed in normal respiration. Moreover, in normal respiration the quantity of material consumed decreases as the plant adjusts itself to the conditions, while in anaerobic respiration the quantity increases. In a general way, therefore, the experiments reported in this paper and those reported in former papers seem to give some support to Boysen-Jensen's theory in so far as the production of cane sugar is greatest under conditions of greatest utilization of material by respiration.

CONCLUSIONS

Under gas pressure of 5 atmospheres or more sweet potatoes are killed. In the killed tissues starch hydrolysis is greatly depressed or inhibited. Cane sugar is converted by hydrolysis into reducing sugars which accumulate.

Starch hydrolysis and cane sugar formation in the sweet potato proceed in the absence of oxygen in the same manner as in air or in an atmosphere of oxygen. The presence of oxygen is therefore not always a necessary condition for the formation of cane sugar in plant organs.

The quantity of material consumed in a given period of time in anaerobic respiration by the sweet potato is greater than the quantity consumed in normal respiration at the same temperature. The actual carbon-dioxid output is also greater under anaerobic conditions. Cane sugar appears not to be consumed in either process.

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INFLUENCE OF HUMIDITY UPON THE STRENGTH AND THE ELASTICITY OF WOOL FIBER[1]

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INTRODUCTION

Much work has been done on the study of the fiber, the yarn, and the finished cloth of wool. It has long been known that wool absorbs moisture from the air, but the first real research along this line appeared by Schloesing (*7*)[2] in 1893. This work was upon the relation of the moisture content of clean wool to the humidity of the air. In 1905 Hartshorne (*2*) published work along this line, the results of which were in substantial agreement with the work of Schloesing. Hartshorne (*3*) formulated his results into the "The laws of regain in cotton and worsted," using these laws in the construction of tables showing the moisture content of wool for a wide range of moisture and temperature conditions of the atmosphere. These tables show the great delicacy with which wool responds to the changes in the relative humidity of the air, and also makes it easy to find the moisture content of wool where the relative humidity of the air is known. He has continued his work upon the regain of worsted and of cotton and is to-day one of our greatest authorities along this line.

The effect of moisture on the strength and elongation of yarns and fabrics was reported by Barker, Barbrick, and Pickles (*1*). In their tests on worsted yarn they found that on increasing the moisture content from "absolute dryness" to saturation there was a decrease in strength but an increase percentage of elongation. They also found that when like patterns of worsted were tested in a room of 92 per cent humidity and then in a room with a humidity of 76 per cent there was an increase in strength and a decrease in elongation. They further found that yarns or fabrics made of cotton increased in both strength and elongation on the increase of the humidity of the surrounding atmosphere.

[1] Approved for publication in the Journal of Agricultural Research by the Director of the Agricultural Experiment Station of the University of Wyoming.

[2] Reference is made by number (italic) to "Literature cited," p. 294-295.

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Lewis (*5*) made tests on woolen and worsted yarns, similar to those of Barker and his coworkers, under controlled conditions of temperature and humidity, at five different humidities ranging from 45 to 85 per cent. He found an increase of 16 per cent in the tensile strength of cotton and a decrease of 18 per cent in the tensile strength of worsted for a rise of 40 per cent in the relative humidity.

The work carried on at the Wyoming Experiment Station in 1911 under the direction of Hill (*4*) showed that the dry wool fiber was stronger than the wet fiber, and that at a humidity of approximately 15 per cent the wool fiber was stronger than at 35 per cent. Because of the lack of the means of temperature and humidity control, this work was temporarily suspended until such control conditions might be established.

EXPERIMENTAL WORK

On undertaking research studies upon wool the writer found that it was first necessary to improve further the means of measuring the strength of the wool fiber before a continuation of studies in the effects of chemical reagents and of alkali and weathering could be made with satisfactory results. In September, 1917, the writer succeeded in bringing a small inside room under automatically controlled conditions of temperature and humidity. A description of this room will be found elsewhere in this article. The work of Hill (*4*) who tested over 59,000 fibers, clearly showed that it was quite impossible to get satisfactory results by testing the single wool fibers under ordinary room conditions. He states (p. 123):

> The variation of the means of hundreds is so great that the mean of this or a smaller number of tests is a very inaccurate measure of the mean of a sample of wool containing only a few thousand fibers, and that the means of thousands can scarcely be used for anything more than the most general work.

Anyone who has tested textile fibers knows that to test only 500 wool fibers is not only a long but a tedious operation, and it would be impracticable to test many samples, were so many tests required for each sample. It was thought, however, that possibly under controlled conditions of temperature and humidity the number of fibers necessary to be tested on each sample, with satisfactory results, might be greatly reduced. With this thought in mind the writer began the work covered in this paper, with a plan outlined to test samples of wool fibers at five humidities ranging from 40 to 80 per cent. Samples of wool from the shoulders of four sheep, a Rambouillet, an Oxford, a Cotswold, and a Dorset were selected. All tests were made upon single fibers from locks of wool which had not been cleaned or scoured. The tests were all made on a Reeser and Mackenzie fiber-testing machine, a machine devised by Matthews, of the Philadelphia Textile School, and fully described in Matthews's Textile Fibers (*6, p. 254*).

In these experiments and all subsequent work the temperature was kept at 70° F., the humidity only being changed. The breaking strengths of the 200 fibers were determined on each sample, first at a humidity of 40, and later at a humidity of 70 per cent. The results of this work are shown in Table I.

TABLE I.—*Breaking strength of wool fibers at two humidities*

Sample No.	Breed.	Relative humidity, 40 per cent.			Relative humidity, 70 per cent.		
		Average of—		Variation between 100 and 200.	Average of—		Variation between 100 and 200.
		100.	200.		100.	200.	
		Dgm.	*Dgm.*	*Per cent.*	*Dgm.*	*Dgm.*	*Per cent.*
991	Rambouillet....	70.82	71.04	0.62	63.14	62.68	1.50
991	.....do.........	71.26	....	....	62.22	....	....
994	Oxford.........	149.49	163.30	15.59	150.74	152.80	2.65
994	.....do.........	177.11	....	....	154.86	....	....
996	Cotswold.......	169.86	178.80	9.56	182.00	178.70	3.63
996	.....do.........	187.81	....	....	175.40	....	....
997	Dorset.........	148.14	140.18	10.74	130.44	130.73	0.44
997	.....do.........	132.22	....	....	131.01	....	....

An examination of Table I, shows that with the increasing of the humidity the breaking strength of the fibers decreases. It will also be noted that the percentage variation between the average breaking strengths of each hundred fibers reaches in one case practically 16 per cent. Had a larger number of fibers been broken, it is probable that the extreme variations between hundreds would have been even greater.

Determining the breaking strength of the fibers under controlled conditions of temperature and humidity is more accurate than under ordinary room conditions; yet the wide variations among the sizes of the individual fibers makes it quite impossible to obtain a small percentage variation between the means of each hundred fibers tested without taking into consideration the diameter of the fibers. An attempt was made to measure the diameter of the fibers in the testing machine by means of a microscope which could be moved horizontally or vertically by means of a screw adjustment. The work was found very slow and tedious, and it appeared that the fibers did not break at the smallest diameter. The fact that wool fibers are very irregular in shape renders the measurements taken from one side of the fiber very inaccurate. If a microscope can be constructed to view the wool fiber from two different angles at the same cross section, there may be obtained much more accurate results by use of this instrument. It seems that this condition may be obtained by a proper adjustment of mirrors, but to the writer's knowledge no such adjustment has ever been tried.

The next arrangement which suggested itself as a means of measuring the fibers was the use of a micrometer caliper. A micrometer caliper graduated to read in hundredths of a millimeter and having a ratchet stop adjustment can readily be set so that contact upon the fibers is uniform and the fiber is not distorted when the contact is made. This micrometer is substituted in place of the lower jaw of the testing machine (Pl. 48, A) so that the diameters may be measured with the greatest speed and accuracy possible with a micrometer. A small hand lens (not shown in the illustration) was supported in front of the micrometer in order to make it possible to read the diameters of the wool fibers to a thousandth of a millimeter.

The diameters of a number of fibers were measured at as many intervals as possible between the two jaws of the testing machine, after which the fibers were tested. The fibers broke in practically every instance at the place where the micrometer indicated the smallest diameter. A number of fibers were very carefully watched under a hand lens as they were being measured with the micrometer. It was observed that as the contact is being made the oval fibers twist so the measurement is made at the smallest diameter. Human hair and the hair from animals were tested with the same result. This led the writer to believe that he was justified in using the smallest diameters obtained by the use of a micrometer in computing the tensile strength (ratio of breaking strength to area of cross section) of the wool fibers.

Another series of tests was made on the same samples as reported in Table I, but for five relative humidities, 40, 50, 60, 70, and 80 per cent, temperature 70° F. Every wool fiber tested was measured at three places between the jaws of the testing machine. The stretch of each fiber was recorded, together with its breaking strength, and the tensile strength calculated from the diameter of the fiber as found at the smallest point. The results of the measurement of the breaking strength are shown in Table II.

TABLE II.—*Breaking strengths of fibers at five humidities*

Sample No.	Breaking strength at a relative humidity of—									
	40 per cent.		50 per cent.		60 per cent.		70 per cent.		80 per cent.	
	Average of 100.	Average of 200.	Average of 100.	Average of 200.	Average of 100.	Average of 200.	Average of 100.	Average of 200.	Average of 100.	Average of 200.
	Dgm.	*Dgm.*	*Dgm.*	*Dgm.*	*Dgm.*	*Dgm.*	*Dgm.*	*Dgm.*	*Dgm.*	*Dgm.*
991	66.82	67.17	65.39	69.92	67.50	69.45	54.67	55.85	55.36	53.85
991	67.51	....	66.44	....	71.40	....	57.02	....	52.34	....
994	182.07	187.49	140.19	146.26	145.12	135.77	159.43	162.12	120.89	129.93
994	194.70	....	152.33	....	126.42	....	164.80	....	138.96	....
996	172.59	195.18	196.67	206.21	248.88	229.00	210.79	200.72	192.71	175.75
996	217.77	....	215.74	....	209.12	....	190.64	....	158.79	....
997	124.53	125.12	102.45	105.44	102.06	105.29	104.10	106.25	103.30	100.20
997	125.70	....	108.42	....	108.53	....	108.35	....	97.10	....

TABLE III.—*Diameter, breaking strength, and tensile strength of wool fibers at five different humidities*

Sample No.	Relative humidity of—																			
	40 per cent.				50 per cent.				60 per cent.				70 per cent.				80 per cent.			
	Diameter, thousandths of a mm. (average of 100).	Breaking strength (average of 100).	Tensile strength, per square hundredths of a mm. (average of 100).	Average of 200.	Diameter, thousandths of a mm. (average of 100).	Breaking strength (average of 100.)	Tensile strength, per square hundredths of a mm. (average of 100).	Average of 200.	Diameter, thousandths of a mm. (average of 100).	Breaking strength (average of 100).	Tensile strength, per square hundredths of a mm. (average of 100).	Average of 200.	Diameter, thousandths of a mm. (average of 100).	Breaking strength (average of 100).	Tensile strength, per square hundredths of a mm. (average of 100).	Average of 200.	Diameter, thousandths of a mm. (average of 100).	Breaking strength (average of 100).	Tensile strength, per square hundredths of a mm. (average of 100).	Average of 200.
		Mgm.	*Mgm.*	*Mgm.*		*Mgm.*	*Mgm.*	*Mgm.*		*Mgm.*	*Mgm.*	*Mgm.*		*Mgm.*	*Mgm.*	*Mgm.*		*Mgm.*	*Mgm.*	*Mgm.*
991	15.5	6,751	357.7	363.1	16.1	6,539	325.2	323.8	16.9	6,750	280.6	290.2	16.0	5,702	283.6	276.1	15.7	5,234	270.4	278.2
991	15.2	6,682	368.5		16.2	6,644	322.4		18.0	7,140	299.7		16.1	5,467	268.6		15.7	5,536	286.0	
994	27.7	19,470	323.1	323.0	23.7	14,019	317.7	315.3	24.1	12,642	277.2	275.3	28.6	16,480	256.6	264.5	25.8	13,896	265.8	255.1
994	26.8	18,208	322.8		24.9	15,233	312.8		26.1	14,512	273.4		27.3	15,943	272.4		25.1	12,089	244.3	
996	29.1	21,777	327.4	332.7	29.2	19,667	293.7	300.5	30.3	20,912	290.1	278.1	29.3	19,064	282.8	283.8	29.8	19,271	276.3	269.0
996	25.5	17,259	337.9		29.9	21,574	307.3		34.5	24,888	266.2		30.8	21,079	282.9		27.8	15,879	261.6	
997	25.2	12,453	249.7	242.3	23.0	10,245	246.6	243.1	25.6	10,853	212.5	219.1	25.5	10,835	212.1	213.0	26.6	10,330	185.9	189.5
997	26.1	12,570	235.0		24.0	10,842	239.7		24.0	10,206	225.6		24.9	10,410	213.9		25.3	9,710	193.2	
Average				315.3				295.7				265.7				259.3				242.9

Table II shows what a large variation may occur in the averages of the breaking strengths of 100 fibers. In the case of No. 991, the fibers are fairly uniform, and there is less variation. The variations are so great in most cases that one would not be justified in making any final deductions from the results. The differences which occur in the breaking strengths of different fibers in the same sample may be more clearly seen by comparing the results of humidities 40 and 70 in Table II with similar

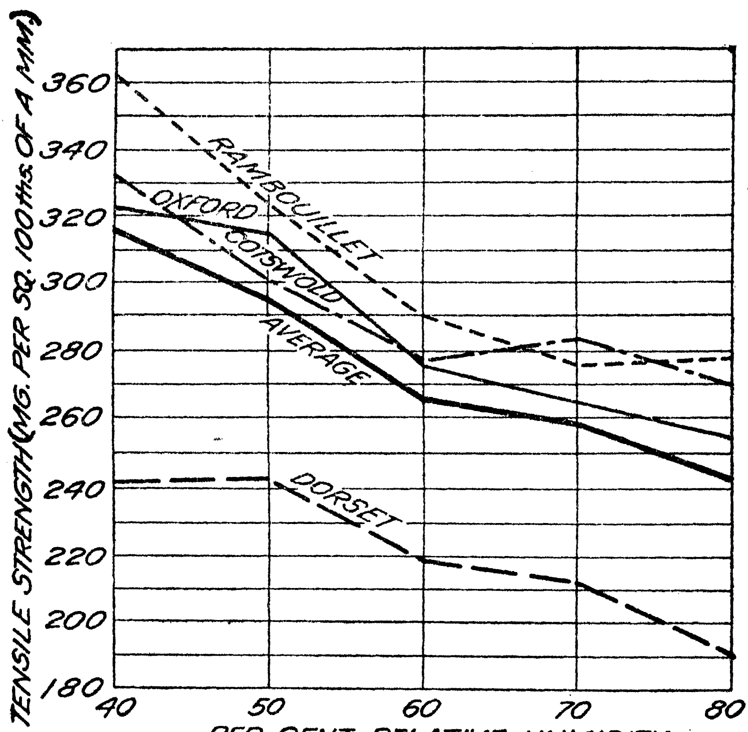

FIG. 1.—Graphs showing the effect of humidity upon the tensile strength of the wool fiber.

results upon the same sample as shown in Table I. Such large variations will occur when the size of each fiber is left out of consideration.

Table III shows the average diameter, breaking strength, and tensile strength for 100 fibers and the average tensile strength for 200 fibers. It can readily be seen that when the diameters of the individual fibers are taken into consideration there is much more uniformity in the results obtained. Had 500 fibers been tested, it is no doubt true that the average tensile strength would have been somewhat more accurate than when only 200 fibers were tested. In the case of sample 991 (humidity 70) 600 fibers were broken, and the average tensile strengths for each

hundred tested were, respectively, 269, 273, 275, 280, 283, and 288. The smallest and largest averages obtained from any two of these figures are 271 and 286, respectively. If each sample is taken into consideration, it will be observed that the average tensile strength is greater in every case at a humidity of 40 than at 60, 70, or 80 per cent. It will also be noted that the average tensile strength of every sample is greater at a humidity of 50 than at 70 or 80, and at 60 it is greater than at 80 per cent. The tensile strength decreases with the increase in the humidity, although in some cases there may be a slight variation up or down when the sample tested is compared with the one tested at the next higher or lower humidity. The average tensile strength of four samples at the different humidities gives figures which show a direct ascent as the percentage of relative humidity is reduced. It would seem that if an average of the four samples was taken, the effects of humidity upon the tensile strength of the wool fiber could be more clearly seen. Graphs of these averages are given in figure 1.

It is again clearly noted that there is a direct increase in the tensile strength of the wool fiber as the relative humidity is reduced, and vice versa. The presence of more yolk on one fiber than on another would make an added variation, as would also the percentage error in the measurement of the fibers.

The percentage elasticity of these four samples was determined at the same time as their breaking strengths, the results being given in Table IV. These tests show that the wool fiber increases in elasticity as the humidity increases. Figure 2 shows curves plotted from the average elasticity of each of the four samples for each humidity, together with the average of all.

It seems probable that each sample would show a curve in closer agreement with that of the final average of figure 2 had 500 or 1,000 fibers been broken upon each sample at the different humidities.

TABLE IV.—*Percentage elasticity of wool fibers at five humidities*

Sample No.	Number of fibers tested.	Elasticity at a relative humidity of—				
		40 per cent.	50 per cent.	60 per cent.	70 per cent.	80 per cent.
		Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
991	200	28.02	31.74	33.67	34.88	35.52
994	200	30.78	33.32	36.92	39.60	42.41
996	200	32.76	38.38	40.24	41.26	47.02
997	200	19.68	26.30	26.44	28.64	31.38
Average		27.81	32.44	34.32	36.10	39.08

The present paper is a progress report, and further humidity studies are being made, both with raw and clean wool.

TEMPERATURE AND HUMIDITY CONTROL

The question of automatically controlling the temperature and humidity is perplexing to the Experiment-Station worker whose funds

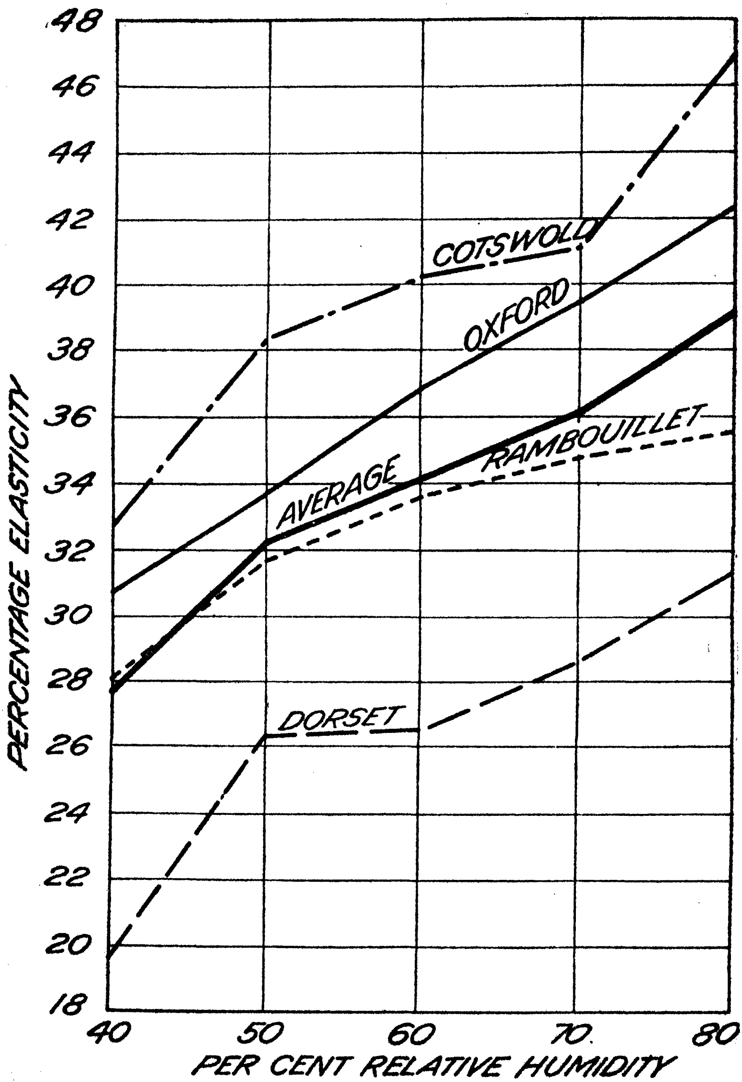

FIG. 2.—Graphs showing the effect of humidity upon the elasticity of the wool fiber.

are limited. The humidity room operated by the writer is simple in its method of control and can be readily installed at a minimum cost.

The room used is an inside one, 6 feet wide, 6 feet long, and 12 feet high. The walls are made of hollow 4-inch gypsum blocks plastered

on both sides, and the ceiling and floor of reinforced concrete. In one side there is a large, well weather-stripped window, the upper part of which has an opening 7 inches wide and extending the entire width of the window. This opening was designed for ventilation purposes, but was found inefficient and was displaced by artificial ventilation. The entrance to the room is provided with double doors separated from each other by a small vestibule, so that one can enter this vestibule and close the door before entering the humidity room. The joints of these doors are well weather-striooed. A corner of the room is shown in Plate 48, A.

The temperature of the room is controlled by a thermograph connected through a pony relay to a bank of lamps fastened overhead and covering an area of about 6 square feet. The lamps remain lighted until the arm of the thermograph records the desired temperature. At this point the indicator of the thermograph makes a contact with a small adjustable platinum arm, thereby closing the circuit from a bell-ringing transformer, which in turn actuates the relay magnet and breaks the light circuit. There is a large tank in the upper part of the room which may be filled with running water and used as a cooler to keep the temperature of the room from going above that desired. At this station, however, it is not ordinarily necessary to use the cooler, as the main laboratory can easily be kept below 70° F., the temperature at which the fiber-testing machine is most used.

The humidity of the room is controlled by an electrical connection through a hydrograph indicator similar to that through the thermograph. When the humidity of the room reaches the desired percentage, as recorded on the hydrograph, the circuit through a $\frac{1}{20}$ h. p. motor which works an atomizer above the tank in the upper part of the room is automatically broken. By means of reducing gears and a crank arm, this motor operates two small air compressers of the bicycle foot-pump type. The two pumps are placed in a horizontal position with their piston rods connected to each other and are also connected through a jointed arm, to the crank pin so that each half turn of the crank causes a forward stroke of one piston and a backward stroke of the other. The air from each pump is conducted to an atomizer in the top of the room. These atomizers are of the household type, but have been modified to fit 1-gallon glass jugs. The greater part of the spray from these atomizers settles into the large water tank, any spray reaching the center of the room being so fine that it is practically all absorbed by the atmosphere before it reaches the floor. The method of pumping is entirely improvised and could easily be replaced by a small electric blower.

Both the thermograph and hydrograph can be quickly set for a new temperature or humidity. The temperature can be regulated with ease at any temperature between 65° and 80° F. and the humidity anywhere

from 35 to 85 per cent. The writer hopes, with certain additions to the room, to make it possible to regulate the humidity at any point between 10 and 90 per cent.

This humidity room has been in constant operation for over seven months, and has proved very satisfactory. It is possible to get a more elaborate equipment and no doubt a more satisfactory one for a larger room, such, for example, as the one in use at the Bureau of Chemistry of the United States Department of Agriculture (*8*), but for a small room and with a comparatively small investment the present arrangement is all that could be desired.

Records of the temperature and the humidity for one week are shown in Plate 48, B. The temperature can easily be regulated at 70° F., with a maximum variation of about 1 degree. The variation in the percentage relative humidity may be regulated to within 2 per cent on the bench where the samples are stored and measured, provided the desired percentage is not over 70. Above this point there is a somewhat larger variation when the door of the humidity room is first opened.

SUMMARY

(1) The breaking-strength determination as a measure of the strength of wool is unsatisfactory because of the wide variations in the size of the individual fibers.

(2) The microscope was found an ineffective means of making a correction for the diameter of the fibers. A micrometer substituted in place of the lower jaw of the testing machine proved to be very efficient in making this correction and reducing the breaking strength to tensile strength or unit stress.

(3) Comparisons of the tensile strengths at five relative humidities—namely, 40, 50, 60, 70, and 80 per cent—showed that the tensile strength of raw wool from four different breeds of sheep decreases as the humidity increases.

(4) Controlled conditions of temperature and humidity were obtained by means of electrical connections through a thermograph and a hydrograph, operating, respectively, a bank of lamps and two atomizers.

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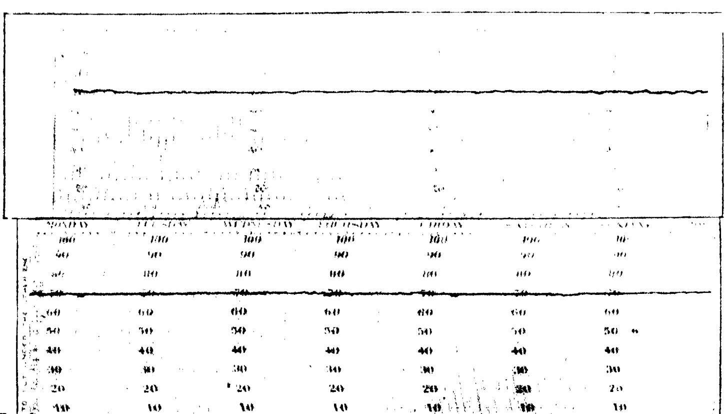
PLATE 48

A—A corner of the humidity room used to test wool fiber.
B—Records of the temperature and humidity during the experiment.



AVAILABILITY OF POTASH IN SOME COMMON SOIL-FORMING MINERALS—EFFECT OF LIME UPON POTASH ABSORPTION BY DIFFERENT CROPS

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INTRODUCTION

Little direct information is obtainable regarding the relative availability of potash carried in common soil-forming minerals. The data found are decidedly contradictory. They have either been obtained from the ability of weak solvents to remove potassium or have been adjudged from the resulting optical properties of the minerals after years of subjection to the forces of weathering.

Numerous petrographic analyses of the soils of the United States (*McCaughey and Fry, 1913*)[1] show that only four minerals which carry potash are found in the very fine sand and coarse silt separates. These are biotite, muscovite, orthoclase, and microcline. In many of the residual soils, such as the Porter and Cecil series (*Plummer, 1915*), the micas are found in large quantitites, and must supply much of their potash. Some of the transported soils, such as those of the Atlantic Coastal Plain, carry comparatively little mica, but often are well supplied with microcline and orthoclase.

It has been known for a good many years that certain neutral salts when in contact with the mineral portion of the soil cause an exchange of bases between the salt and soil. Owing to this action, many claims have been made regarding the effects of lime and other compounds for increasing the soluble potash of the inert soil mass. More recent experiments give indications that the effect of lime and gypsum in bringing into solution potash from the mineral portion of the soil is slight or nil. None of these investigations, however, as shown by the following brief review, have thoroughly covered the direct action of lime compounds on those minerals which supply the soil with potassium.

REVIEW OF PREVIOUS INVESTIGATIONS

So far as the writer is aware, Johnstone (*1889*) was the first to report on the stability of micaceous minerals. This investigator found that, after suspension of mica for as much as one year in carbonated water, no alteration could be detected.

Hilgard (*1906, p. 51*), in speaking of soils formed from mica schist, says:

> . . . mica schist, which being a mixture of quartz and mica only, not only weathers very slowly, but also supplies but little of any importance to plants to the soils formed from it.

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 314-315.

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Hartwell and Pember (*1908*) conducted experiments with feldspar (variety not given) as the source of potash for plants. Their results led to the conclusion that little could be expected from this material as a source of available potash.

Prianischnikow (*1912*) experimented with a number of crops, using various minerals as the source of potash. From his work conclusions are drawn that biotite and muscovite are superior to feldspar (orthoclase and microcline) as carriers of potash.

Fraps (*1912*) found that all potash is extracted from biotite with strong hydrochloric acid, about one-third from muscovite, and only a small percentage from orthoclase and microcline. Fraps also found that practically no potash is removed from orthoclase and microcline by *N/5* nitric acid, less than 10 per cent from biotite, and 15 per cent from muscovite.

McCaughey and Fry (*1913*) conclude from observations of the optical properties of the potash-bearing soil-forming minerals that biotite must give up its potash to solution faster than muscovite and orthoclase faster than microcline.

Curry and Smith (*1914*) found from fertilizer experimentation for hay that calcium carbonate and lime have practically no effect on the solubility of soil potash.

Plummer (*1915*) found indications from field experiments that soils with high content of the micas respond less to potash fertilization than do those in which the feldspars predominate.

Clark (*1916, p. 395*) says:

> Muscovite under ordinary circumstances is one of the least alterable of minerals. The feldspar of a granite may be completely kaolinized, while the imbedded plates of mica retain their brilliancy unchanged.

Lyon and Bizzell (*1916*) say, as a result of lysimeter experiments:

> So far as could be ascertained from the potassium in the drainage water and the crop raised on the soil treated with lime and the soil not so treated, there was no liberation of potassium effected by the lime treatment.

Fraps (*1916*) finds only slight gains of potash due to additions of carbonate of lime on the insoluble potash of the soil.

Briggs and Breazeale (*1917*) find that calcium-hydrate solutions do not modify the solubility of potash in orthoclase or orthoclase-bearing soils.

In view of the variance of results set forth in the foregoing discussions, it would seem desirable that experimentation be carried out to determine the relative availability of potash in the minerals which supply the soil with this constituent.

EXPERIMENTAL WORK

The minerals used were as representative and free from impurities as could be obtained. Each specimen was ground to an impalpable powder and sifted through the finest grade of bolting cloth.

Table I gives the composition of the materials used in this work.

TABLE I.—*Composition of materials used*

Material.	Nitrogen (N).	Available phosphoric acid (P_2O_5).	Potash (K_2O).	Lime (CaO).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*
Dried blood	13.70			
Acid phosphate		16.20		(a)
Potassium sulphate			50.80	
Biotite			8.45	(a)
Muscovite			9.14	(a)
Orthoclase			13.40	(a)
Microcline			14.40	(a)
Precipitated calcium carbonate				56.02

[a] Not determined.

The potash contents, which are very close to the theoretical for the individual minerals, indicate specimens of exceptional purity. Petrographic examinations of the feldspars give all characteristic optical properties of pure orthoclase and microcline, respectively.

SOLUBILITY OF MINERAL POTASH IN CARBONATED WATER AND THE EFFECT OF CALCIUM BICARBONATE THEREON

Water charged with carbon dioxid is generally considered the chief solvent of inert plant nutrients of the soil. To obtain the true availability of any dormant constituent, several extractions are necessary—that is, until a point is reached at which no appreciable amount goes into solution.

Calcium bicarbonate results from the presence of any basic calcium compounds in the soil, and is the form of lime which naturally functions in the exchange of bases.

For comparison with soil conditions, carbonated water and calcium bicarbonate were selected for measuring the availabilty of potash in the four soil-forming minerals.

Distilled water was saturated under pressure with carbon dioxid. The solution of calcium bicarbonate [$Ca(HCO_3)_2$] was *N/20* in strength, and contained an excess of carbon dioxid to prevent the precipitation of calcium carbonate ($CaCO_3$). Thirty gm. of each material and 200 cc. of the solvent were placed in 500-cc. flasks and agitated in an end-over-end slaking machine for 96 hours. At the end of this time suspended matter was allowed to settle, the solutions were clarified, and potash was determined colormetrically, according to methods given by Schreiner and Failyer (*1906*). The residue was thrown on a filter and washed free of potash, after which it was again extracted as before, and the process repeated four times.

The results obtained will be found in Table II.

TABLE II.—*Solubility of potash in common soil-forming minerals, with the effect of calcium bicarbonate*

[Results expressed as parts per million of potassium oxid]

Mineral.	Amount taken.		Extractions with distilled water.						Extractions with carbonated water.						Extractions with calcium bicarbonate in carbonated water.						Gain or loss for calcium bicarbonate.
	Gm.	*Cc.*	1	2	3	4	5	Total.	1	2	3	4	5	Total.	1	2	3	4	5	Total.	
Biotite	30	200	11.0	6.1	5.6	1.2	1.0	24.9	116.0	87.0	49.6	16.0	5.6	274.2	108.0	92.0	44.0	10.0	6.4	251.4	−22.8
Do	30	200	12.6	6.0	5.0	3.1	.6	27.3	109.0	84.0	45.7	11.4	8.2	258.3	110.0	80.0	41.0	15.0	7.9	253.9	− 4.4
Do	30	200	10.0	7.9	5.4	1.0	.6	24.9	111.0	80.0	40.2	14.3	6.8	252.3	108.0	85.0	42.0	13.4	7.0	255.4	+ 3.1
Do	30	200	10.0	8.0	6.2	4.0	.2	28.9	108.0	89.0	43.0	13.6	6.6	260.2	114.0	90.0	46.0	14.0	5.2	269.2	+ 9.2
Average			10.9	7.0	5.5	2.3	.6	26.3	111.0	85.0	44.6	13.8	6.8	261.3	110.0	86.8	43.3	13.1	6.6	257.5	− 3.8
Muscovite	30	200	13.6	6.0	4.0	2.4	.4	26.4	89.4	40.0	26.0	11.1	4.0	170.5	92.0	43.0	24.0	12.0	3.9	174.9	+ 4.4
Do	30	200	11.4	5.0	3.8	1.8	.3	22.3	88.0	41.2	24.7	12.6	2.0	168.5	90.0	39.0	23.0	10.9	3.2	166.1	− 2.4
Do	30	200	10.2	5.4	6.2	.8	.7	23.3	89.0	41.0	24.7	13.6	3.2	170.9	86.0	46.0	27.0	13.0	2.4	174.4	+ 3.5
Do	30	200	10.0	6.0	6.0	1.2	1.8	25.0	86.0	41.0	26.3	12.0	2.4	167.8	86.0	40.0	25.0	11.8	3.9	166.7	− 1.1
Average			11.3	5.6	5.0	1.5	.8	24.2	88.1	40.8	25.4	12.2	2.9	169.4	88.5	42.0	24.8	11.8	3.5	170.5	+ 1.1
Orthoclase	30	200	10.6	6.6	4.0	2.0	.6	23.8	41.8	21.4	17.6	12.4	4.6	97.8	44.0	20.0	18.6	14.2	5.0	101.8	+ 4.0
Do	30	200	8.9	5.4	3.8	1.6	.4	20.1	37.3	21.0	16.0	11.7	5.6	91.6	40.0	21.6	15.0	10.0	4.2	90.8	− .8
Do	30	200	9.4	5.6	2.6	.6	.3	18.4	35.3	23.0	17.6	11.7	5.0	92.6	39.0	26.0	16.0	10.0	6.4	97.7	+ 4.8
Do	30	200	9.4	5.0	3.0	1.2	.6	19.2	......	......	......	......	......	......	33.0	23.0	12.0	12.0	6.4	[a] 86.4	.....
Average			9.7	5.7	3.4	1.4	.5	20.4	38.1	16.4	17.1	11.9	5.1	94.0	39.0	22.6	15.4	11.3	5.5	96.7	+ 2.7
Microcline	30	200	10.0	6.0	2.0	.8	1.0	19.8	30.6	16.0	7.9	6.6	3.0	64.1	28.0	16.6	8.4	5.8	3.8	62.6	− 1.5
Do	30	200	11.3	4.1	2.4	.6	.4	18.8	25.6	17.6	8.2	6.0	4.8	62.2	30.0	19.4	7.0	6.1	5.3	67.8	+ 5.6
Do	30	200	8.0	5.0	1.6	1.2	.2	16.0	26.6	15.8	8.2	5.6	5.0	61.2	25.4	15.0	8.0	5.6	4.6	58.6	− 2.6
Do	30	200	10.0	5.0	2.0	.9	.1	18.0	23.3	17.6	8.0	6.4	2.8	58.1	22.0	14.8	7.6	6.6	3.8	54.8	− 3.2
Average			9.8	5.0	2.0	.9	.4	18.1	26.5	16.8	8.1	6.1	3.9	61.4	26.4	16.4	7.8	6.0	4.4	60.9	− .4

[a] Not included in average.

The results obtained from these experiments indicate only small differences in the solubility of potash in distilled water. Biotite and muscovite appear to give up somewhat more of this plant nutrient to water than do the feldspars. These differences are small, however, and may be due to experimental error.

With carbonic acid as the solvent the divergence in the amounts of potash going into solution is more marked. More than four times as much potash of biotite is dissolved as is carried by microcline. Muscovite stands next to biotote in the solubility of its potash, and orthoclase is slightly ahead of microcline.

These findings agree rather closely with the vegetative experiments detailed later and follow the same order as those of Fraps (*1912*), in which a weak solution of nitric acid was used as the solvent.

Calcium bicarbonate has not shown any power to unlock potash from any of the minerals. With biotite and microcline there are slight losses of potash when this material is used in connection with water charged with carbon dioxid. Only very small gains from the use of the bicarbonate are discernible with muscovite and orthoclase, gains so small as to be considered negligible and to have no practical significance.

Briggs and Breazeale (*1917*) have recently reached the same conclusions from the use of calcium hydroxid and gypsum on orthoclase and certain orthoclase-bearing soils.

VEGETATIVE EXPERIMENTS WITH THE COMMON SOIL-FORMING MINERALS

The solubility investigations just given have shown rather marked differences in the power with which potash is held in the two micas and feldspars. For the purpose of supplementing the laboratory data, pot experiments were begun in which four different crops were grown out of doors to maturity. These were oats (*Avena sativa*), soybeans (*Soja max*), rye (*Secale cereale*), and cowpeas (*Vigna sinensis*).

Description of Pot Experiments

SOIL USED

The soil used in this investigation was taken from the no-treatment plots of the Edgecombe (N. C.) Branch Station, where experiments to determine its fertilizer requirements have been running for the past 15 years. The field tests (*Kilgore et al., 1914*) indicate rather conclusively that potash is one of the limiting elements of this soil; also that the available plant nutrients have been reduced to a minimum on the plots receiving no additions. The soil was taken from the plots to a depth of 6⅔ inches. Tables III and IV give the chemical and mineralogical composition of the soil used.

TABLE III.—*Chemical composition of soil used*

Plant nutrient.	Percentage composition of oven-dried soil.	Quantity per acre of 2,000,000 pounds of soil.
		Pounds.
Nitrogen	0.032	640
Phosphoric acid	.026	520
Potash	.094	1,880
Soda	.041	820
Lime	.154	3,080
Magnesia	.082	1,640

TABLE IV.—*Petrographic analysis of soil*

Percentage of minerals not quartz in—		Abundant minerals not quartz in—		Less abundant minerals not quartz in—		Remarks.
Sand.	Silt.	Sand.	Silt.	Sand.	Silt.	
2–4	5–8	None	None	Orthoclase (residues), microcline, epidote, tourmaline, magnetite, hornblende.	Epidote, tourmaline, zircon, rutile, magnetite, sillimanite, hornblende, muscovite, biotite, garnet.	Soil characterized by low content of minerals other than quartz. Only trace of mica present. Minerals existing are of a refractory nature.

CONDITIONS OF PLANT GROWTH

The equivalent of 40 pounds of oven-dried soil was carefully weighed out, the various plant nutrients were added in the amounts given in Table V and were mixed thoroughly by rolling over and over on canvas cloth. This was transferred to 4-gallon glazed earthenware pots. Sufficient drainage was obtained through small openings on the lower side of each pot.

Nitrogen and phosphoric acid were added to all pots two weeks before seeding each crop. Potash and lime were added only at the beginning of the experiment.

The rates of application were made on the basis of 200 and 400 pounds of potash per acre. For convenience of expressing the data obtained, in Tables V to X the treatments are referred to as the mineral which carries potash in weights of 200 pounds per acre. The figure "2" before the name of the potash carrier indicates that this plant nutrient has been applied at the rate of 400 pounds per acre.

This work was conducted out of doors in a cage of ¼-inch-mesh poultry wire. Excessive heat is prevented in summer by a lattice-work cover similar to those used in covering ginseng beds. During spring and summer the pots were placed on benches 2 feet above the surface of the ground. In winter they were buried in a mixture of sawdust and soil sufficiently deep to prevent freezing.

TABLE V.—*Rate of application of plant nutrients*

Carrier.	Quantity of carrier per pot.	Quantity of plant nutrients (pounds per acre of 2,000,000 pounds of soil).			
		Nitrogen.	Phosphoric acid.	Potash.	Lime.
	Grams.				
Dried blood	4. 834	73	......	......	....
Acid phosphate	13. 424	......	224	......	(a)
Potassium sulphate	3. 570	......	......	200	....
2 potassium sulphate	7. 140	......	......	400	....
Biotite	21. 488	......	......	200	(a)
2 biotite	42. 976	......	......	400	(a)
Muscovite	19. 872	......	......	200	(a)
2 muscovite	39. 744	......	......	400	(a)
Orthoclase	13. 548	......	......	200	(a)
2 orthoclase	27. 096	......	......	400	(a)
Microcline	12. 552	......	......	200	(a)
2 microcline	25. 104	......	......	400	(a)
Precipitated calcium carbonate	33. 420	......	......	......	2, 000
2 precipitated calcium carbonate	66. 840	......	......	......	4, 000

a Not determined.

Enough water was added each day, when necessary, to keep the soil well moistened during periods of plant growth.

Acid-washed quartz was placed over each pot after seeding to act as a mulch.

OAT CROPS

On March 24, 1916, 20 seeds of the Burt variety of oats were planted to each pot. After germination the plantlets were drawn down to a uniform stand of 12 per pot. On the following June 10 the oat crop was harvested after reaching maturity. Owing to the inability of removing all the roots, only the portion of the plants above ground was considered.

Potash was determined separately in the grain and straw of each pot. Table VI contains the data obtained.

TABLE VI.—*Weight of oat crop and potash removed from soil*

Treatment.	Dry matter (grams).					Potash removed from soil (grams).				
	Grain.	Straw.	Total.	Average total.	Relative rank of average.	Grain.	Straw.	Total.	Average total.	Gain over no potash.
Potassium sulphate	13. 8	24. 9	38. 7	37. 0	82. 4	0. 065	0. 306	0. 371	0. 363	0. 305
Do	16. 4	22. 4	38. 8			. 083	. 300	. 383		
Do	12. 7	20. 9	33. 7			. 062	. 284	. 346		
2 potassium sulphate	17. 2	27. 6	44. 8	44. 8	99. 8	. 086	. 366	. 452	. 454	. 396
Do	18. 0	24. 9	42. 9			. 088	. 366	. 454		
Do	20. 0	26. 8	46. 8			. 092	. 367	. 457		
2 potassium sulphate plus calcium carbonate	21. 1	28. 0	49. 1	44. 8	99. 8	. 107	. 434	. 541	. 482	. 417
Do	20. 0	25. 0	45. 0			. 092	. 425	. 417		
Do	17. 6	22. 8	40. 4			. 088	. 400	. 488		

TABLE VI.—*Weight of oat crop and potash removed from soil*—Continued

Treatment.	Dry matter (grams).					Potash removed from soil (grams).				
	Grain.	Straw.	Total.	Average total.	Relative rank of average.	Grain.	Straw.	Total.	Average total.	Gain over no potash.
2 potassium sulphate plus 2 calcium carbonate	18.6	24.0	42.6			0.102	0.410	0.512		
Do	19.4	26.0	45.4	44.9	100.0	.087	.422	.409	0.474	0.409
Do	20.9	25.8	46.7			.084	.416	.502		
Biotite	9.9	15.0	24.9			.039	.234	.273		
Do	10.6	18.6	29.2	28.7	63.9	.053	.264	.321	.307	.244
Do	12.4	19.8	32.2			.056	.271	.327		
2 biotite	11.2	17.5	28.7			.058	.241	.299		
Do	13.0	20.6	33.6	30.0	66.8	.062	.262	.324	.299	.234
Do	10.8	16.9	27.7			.040	.236	.276		
2 biotite plus calcium carbonate	9.8	17.8	27.6			.040	.240	.280		
Do	12.6	20.9	33.5	31.0	69.0	.052	.256	.308	.299	.234
Do	10.6	21.4	32.0			.046	.262	.308		
2 biotite plus 2 calcium carbonate	10.6	16.4	27.0			.044	.228	.272		
Do	13.2	18.9	32.1	29.1	64.8	.050	.240	.290	.278	.213
Do	13.0	17.0	30.0			.052	.219	.271		
Muscovite	6.4	14.4	20.8			.029	.218	.247		
Do	6.4	14.0	20.4	21.3	47.4	.029	.200	.229	.303	.238
Do	7.9	15.9	23.8			.031	.224	.255		
2 muscovite	7.0	16.0	23.0			.033	.206	.239		
Do	7.8	16.9	24.7	23.8	53.0	.036	.200	.236	.241	.179
Do	8.0	15.8	23.8			.040	.218	.258		
2 muscovite plus calcium carbonate	6.8	13.6	20.4			.030	.196	.226		
Do	7.1	16.0	23.1	22.3	49.6	.032	.218	.250	.244	.179
Do	7.3	16.0	23.3			.033	.224	.257		
2 muscovite plus 2 calcium carbonate	7.0	15.6	22.6			.029	.230	.259		
Do	6.9	13.8	20.7	21.3	47.4	.026	.206	.232	.240	.175
Do	7.6	13.0	20.6			.031	.109	.230		
Orthoclase	4.2	9.4	13.6			.018	.141	.159		
Do	3.6	8.6	12.2	12.3	27.3	.016	.128	.144	.143	.075
Do	3.6	7.5	11.1			.016	.110	.126		
2 orthoclase	3.8	10.9	14.7			.012	.162	.174		
Do	4.8	10.8	15.6	14.3	31.8	.019	.160	.179	.172	.107
Do	3.0	9.8	12.8			.018	.146	.164		
Do	4.1	9.0	13.1			.020	.154	.174		
Do	2.9	7.8	10.7	12.1	26.9	.010	.126	.136	.148	.073
Do	3.8	8.6	12.4			.016	.120	.136		
2 orthoclase plus calcium carbonate	3.9	8.6	12.5			.018	.118	.136		
Do	3.0	10.0	13.0	13.7	30.7	.018	.156	.174	.165	.100
Do	4.6	11.1	15.7			.022	.164	.186		
Microcline	1.8	6.2	8.0			.009	.083	.092		
Do	.8	5.0	5.8	6.4	14.2	.004	.076	.080	.081	.016
Do	1.0	4.4	5.4			.007	.064	.071		
2 microcline	2.0	5.6	7.6			.008	.068	.076		
Do	1.2	6.6	7.8	6.9	15.4	.006	.084	.090	.075	.010
Do	.6	4.8	5.4			.003	.057	.060		
2 microcline plus calcium carbonate	1.1	5.0	6.1			.004	.072	.076		
Do	2.4	7.0	9.4	7.1	15.8	.007	.088	.095	.080	.015
Do	.9	4.9	5.8			.002	.069	.071		
2 microcline plus 2 calcium carbonate	.5	4.5	5.0			.001	.064	.065		
Do	1.7	4.2	5.9	6.3	14.0	.003	.074	.077	.077	.012
Do	2.2	5.8	8.0			.006	.083	.089		
Control (no potash)	1.6	4.0	5.6			.002	.072	.074		
Do	.5	3.8	4.3	5.1	11.3	.003	.051	.054	.065	....
Do	.5	4.9	5.4			.004	.063	.067		
Control (no potash) plus calcium carbonate	.9	5.0	5.9			.002	.072	.044		
Do	.8	3.2	4.0	4.8	10.7	.002	.056	.058	.058	None.
Do	1.6	2.8	4.4			.003	.041	.074		
Control (no potash) plus 2 calcium carbonate	1.1	4.0	5.1			.002	.066	.068		
Do	.9	3.8	4.7	4.3	9.5	.004	.058	.062	.062	None.
Do	.5	2.8	3.3			.003	.053	.056		

These data show quite conclusively that the oat plant is capable of extracting potash from the soil minerals at different rates. When the greatest yield, 2 potassium sulphate and 2 calcium carbonate, is given the rank of 100, the following order of plant growth is obtained: Two biotite plus 2 calcium carbonate reaches 69; 2 muscovite alone 53; 2 orthoclase alone 31.8; 2 microcline plus calcium carbonate 15.8; and no potash without calcium carbonate 11.3.

This soil responds markedly to potash fertilization, as shown by the oat yields. Soluble potash produces growth to the extent of 44.8 gm. per pot; where no potash material is applied only 5.1 gm. per pot is secured.

To judge from the plant growth, lime has not made available any of the insoluble potash applied in the form of minerals. In many instances the yield has been slightly reduced where the carbonate has been used.

The results of plant growth fertilized with double applications of potash and lime are shown graphically in figure 1.

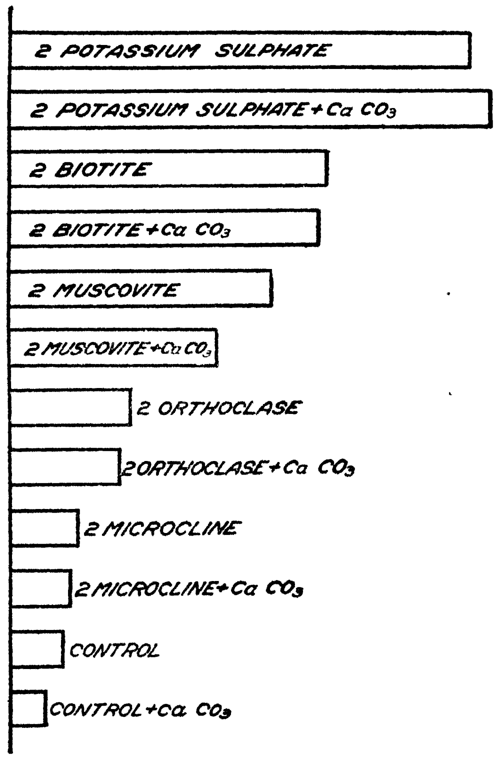

FIG. 1.—Rate of growth of oats under similar conditions fertilized with double applications of potash minerals and calcium carbonate.

Considerably more potash has been recovered in the crop when applied in the soluble form. The order of potash recovery follows the same order as crop yield, biotite showing the greatest and microcline the least. Lime has not increased this recovery in any of the treatments (Pl. 49, A).

Soybean Crop

After harvesting the oats the quartz mulch was removed; the roots of the oats were finely ground and mixed thoroughly with the soil.

All pots were inoculated with as nearly the same number of a pure culture of *Bacillus radicicola* as could be done.

On June 19, 10 seeds of Mammoth Yellow variety of soybean were seeded to each pot. These were drawn down after germination to a

uniform stand of five plantlets per pot. The crop was harvested on September 25, after maturing seed.

In Table VII will be found the results obtained.

TABLE VII.—*Weight of soybean crop and potash removed from soil*

Treatment.	Dry matter (grams).					Potash removed from soil (grams).				
	Seed.	Hay.	Total.	Average total.	Relative rank of average.	Seed.	Hay.	Total.	Average total.	Gain over no potash.
Potassium sulphate	14.8	63.4	78.2			0.160	0.382	0.542		
Do	14.1	60.9	75.0	77.3	63.0	.150	.376	.526	0.545	0.409
Do	15.7	63.0	78.7			.174	.392	.566		
2 potassium sulphate	15.2	68.0	83.2			.271	.526	.797		
Do	16.9	70.0	86.9	85.5	61.5	.280	.531	.811	.802	.666
Do	16.6	70.0	86.6			.279	.520	.799		
2 potassium sulphate plus calcium carbonate	27.7	82.2	109.9			.526	.556	1.082		
Do	26.0	86.3	112.3	112.8	90.8	.482	.562	1.044	1.099	.963
Do	28.2	88.0	116.2			.566	.604	1.170		
2 potassium sulphate plus 2 calcium carbonate	29.8	91.0	120.8			.574	.622	1.196		
Do	33.2	93.0	126.2	124.2	100.0	.642	.650	1.292	1.234	1.098
Do	32.1	93.6	125.7			.569	.646	1.215		
Biotite	9.8	56.1	65.7			.167	.403	.570		
Do	8.7	55.0	63.7	66.1	53.2	.152	.386	.548	.567	.431
Do	10.6	57.9	68.5			.170	.414	.584		
2 biotite	10.3	55.0	65.0			.170	.400	.570		
Do	9.0	52.0	61.0	64.8	52.1	.158	.377	.534	.575	.339
Do	11.4	56.6	68.0			.204	.416	.620		
2 biotite plus calcium carbonate	17.8	70.3	88.1			.307	.490	.797		
Do	16.5	68.6	85.1	88.3	71.1	.286	.462	.748	.791	.655
Do	18.4	72.4	91.8			.324	.504	.828		
2 biotite plus 2 calcium carbonate	16.0	68.2	84.2			.314	.440	.754		
Do	17.4	71.8	89.2	87.8	70.7	.306	.468	.774	.789	.653
Do	17.0	73.0	90.0			.310	.530	.840		
Muscovite	9.4	50.2	59.6			.162	.364	.526		
Do	10.8	51.7	52.5	56.8	45.7	.171	.360	.531	.528	.392
Do	10.0	48.3	58.3			.170	.358	.528		
2 muscovite	11.6	47.6	59.2			.180	.351	.531		
Do	10.8	46.9	57.7	58.7	47.2	.172	.350	.522	.534	.388
Do	10.4	50.2	60.6			.175	.376	.551		
2 muscovite plus calcium carbonate	13.8	58.0	71.8			.234	.418	.652		
Do	12.2	60.0	72.2	73.9	59.5	.226	.420	.646	.663	.527
Do	14.9	63.0	77.9			.245	.446	.691		
2 muscovite plus 2 calcium carbonate	11.9	57.0	68.9			.189	.400	.589		
Do	13.6	59.8	73.4	69.7	56.1	.246	.412	.658	.625	.489
Do	12.0	56.4	66.4			.225	.402	.627		
Orthoclase	6.0	30.6	36.6			.112	.281	.393		
Do	5.6	34.0	39.6	36.5	29.2	.106	.294	.400	.388	.252
Do	4.8	28.6	33.4			.100	.272	.372		
2 orthoclase	5.0	31.0	36.0			.096	.290	.386		
Do	6.6	36.2	42.8	40.4	32.5	.116	.306	.422	.408	.272
Do	6.3	36.2	42.5			.114	.304	.418		
2 orthoclase plus calcium carbonate	9.1	49.2	58.3			.133	.344	.477		
Do	8.6	46.0	54.6	55.3	44.5	.130	.346	.476	.473	.337
Do	7.9	45.1	53.0			.135	.232	.467		
2 orthoclase plus 2 calcium carbonate	7.6	45.3	52.9			.132	.330	.462		
Do	8.4	47.0	55.4	55.6	44.7	.130	.338	.468	.468	.332
Do	9.2	49.4	58.6			.129	.346	.475		
Microcline	3.2	16.1	19.3			.066	.112	.178		
Do	2.6	15.6	18.2	18.0	14.4	.052	.106	.158	.166	.030
Do	2.6	14.2	16.8			.050	.111	.161		
2 microcline	3.4	15.0	18.4			.069	.105	.174		
Do	2.8	13.8	16.6	17.9	14.4	.048	.100	.148	.166	.030
Do	4.1	14.6	18.7			.072	.103	.175		
2 microcline plus calcium carbonate	5.3	19.1	24.4			.078	.114	.192		
Do	6.0	20.7	26.7	25.8	20.7	.080	.119	.199	.197	.061
Do	5.8	20.5	26.3			.076	.120	.196		
2 microcline plus 2 calcium carbonate	5.4	21.6	27.0			.080	.122	.202		
Do	6.3	20.0	26.3	26.5	21.3	.076	.119	.195	.197	.061
Do	6.3	22.0	26.3			.075	.119	.194		

TABLE VII.—*Weight of soybean crop and potash removed from soil*—Continued

Treatment.	Dry matter (grams).					Potash removed from soil (grams).				
	Seed.	Hay.	Total.	Average total.	Relative rank of average.	Seed.	Hay.	Total.	Average total.	Gain over no potash.
Control (no potash)	2.0	11.0	13.0			0.046	0.086	0.132		
Do	2.0	10.8	12.8	13.6	10.9	.053	.072	.125	0.136	......
Do	3.6	12.6	15.2			.062	.091	.153		
Control (no potash) plus calcium carbonate	5.0	17.6	22.6			.065	.100	.165		
Do	4.6	16.0	20.6	21.6	17.3	.058	.096	.154	.161	0.025
Do	4.0	16.6	20.6			.062	.102	.163		
Control (no potash) plus 2 calcium carbonate	4.2	16.0	20.2			.062	.102	.164		
Do	5.6	18.3	23.9	21.8	17.5	.069	.106	.175	.166	.030
Do	4.0	17.4	21.5			.060	.099	.159		

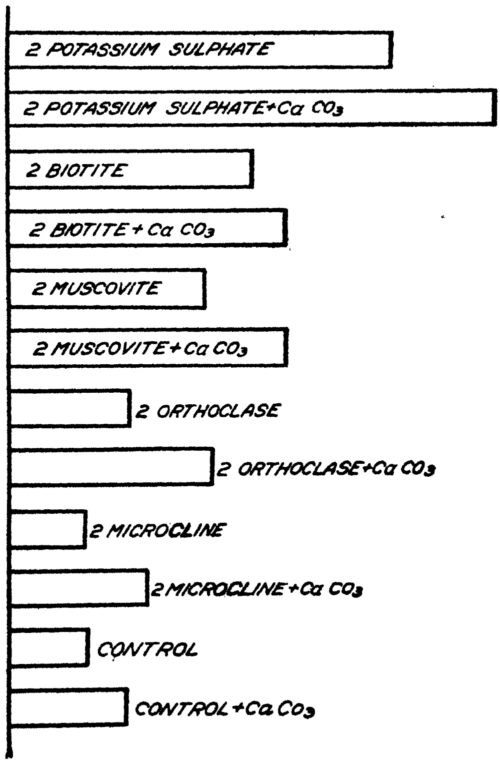

FIG. 2.—Rate of growth of soybeans under similar conditions fertilized with double applications of potash minerals and calcium carbonate.

The results shown in Table VII follow the same order regarding the relative availability of the insoluble potash as with the oat crop. Greatest growth has been obtained from biotite, then muscovite, orthoclase, and the least from microcline.

Calcium carbonate has materially increased plant growth and the potash recovered in the crop when supplied with potassium sulphate, biotite, and muscovite; but to a much lesser extent with the feldspar and where no potash was added. This should not be taken to indicate that lime has been exchanged directly for potash in the applied minerals, but has produced conditions in the soil more favorable to the growth of the legume. In this way hardier plants are produced which are capable of extracting more of this constituent from the minerals in which it is not so securely held.

Figure 2 shows graphically the rates of the growth of soybeans fertilized with double applications of potash and lime. (See also Pl. 49, B.)

RYE CROP

The soybean roots were ground as those of the oats and thoroughly incorporated with the soil. On October 3, 1916, 20 rye seed were added to each pot, of which 12 plantlets were left to mature. On June 4, 1917, the rye was harvested. Table VIII contains the data of this harvest.

TABLE VIII.—*Weight of rye crop and potash removed from soil*

Treatment.	Dry matter (grams).					Potash removed (grams).				
	Grain.	Straw.	Total.	Average total.	Relative rank of average.	Grain.	Straw.	Total.	Average total.	Average gain over no potash.
Potassium sulphate	8.1	29.1	37.2			0.046	0.162	0.208		
Do	7.5	28.0	35.5	36.5	76.5	.040	.151	.191	0.202	0.129
Do	8.8	28.0	36.8			.048	.158	.206		
2 potassium sulphate	10.4	29.9	40.3			.065	.260	.325		
Do	9.6	28.4	38.0	38.5	80.7	.051	.255	.306	.312	.247
Do	9.6	27.6	37.2			.054	.250	.304		
2 potassium sulphate plus calcium carbonate	12.0	30.0	42.0			.069	.271	.340		
Do	14.0	34.0	48.0	46.5	97.4	.074	.274	.358	.349	.276
Do	13.6	36.0	49.6			.076	.275	.351		
2 potassium sulphate plus 2 calcium carbonate	12.8	32.6	45.4			.070	.268	.338		
Do	13.4	37.4	50.8	47.7	100.0	.072	.280	.352	.353	.280
Do	14.4	38.2	52.6			.086	.282	.368		
Biotite	6.4	26.6	33.0			.039	.186	.225		
Do	5.8	26.0	31.0	32.0	67.1	.036	.180	.216	.219	.146
Do	6.0	25.2	31.2			.042	.174	.216		
2 biotite	7.0	27.2	34.2			.040	.194	.234		
Do	6.4	25.0	31.4	32.3	67.7	.039	.174	.213	.222	.149
Do	6.9	24.6	31.5			.044	.175	.219		
2 biotite plus calcium carbonate	6.7	26.0	32.7			.044	.180	.224		
Do	6.6	26.0	32.6	32.0	67.1	.040	.180	.220	.225	.152
Do	5.4	25.4	30.8			.038	.173	.211		
2 biotite plus 2 calcium carbonate	7.2	27.0	34.2			.048	184	.232		
Do	5.7	26.0	31.7	32.2	67.5	.036	.180	.216	.218	.145
Do	5.7	24.9	30.6			.033	.172	.205		
Microcline	2.0	10.4	12.4			.016	.078	.094		
Do	1.6	11.6	13.2	11.7	24.5	.014	.083	.107	.093	.020
Do	.8	8.8	9.6			.009	.069	.078		
2 microcline	1.4	10.0	11.4			.009	.074	.083		
Do	.9	8.8	9.7	9.9	20.7	.007	.062	.069	.071	None.
Do	.9	7.6	8.5			.006	.056	.062		
2 microcline plus calcium carbonate	1.8	8.8	10.6			.008	.066	.074		
Do	.5	7.0	7.5	9.3	19.4	.003	.073	.076	.078	.005
Do	.6	9.2	9.8			.004	.080	.084		
2 microline plus 2 calcium carbonate	1.0	8.8	9.8			.005	.064	.069		
Do	.5	9.0	9.5	9.9	20.7	.002	.069	.071	.073	None.
Do	.9	9.6	10.5			.004	.074	.074		
Control (no potash)	1.6	8.4	10.0			.007	.068	.075		
Do	1.6	8.0	9.6	9.7	20.5	.007	.064	.071	.073	......
Do	.3	7.1	7.4			.001	.071	.072		
Control (no potash) plus calcium carbonate	.3	9.0	9.3			.001	.066	.067		
Do	.6	8.0	8.6	9.3	19.4	.003	.060	.063	.066	None.
Do	1.4	8.6	10.0			.007	.062	.069		
Control (no potash) plus 2 calcium carbonate	1.4	7.6	9.0			.007	.064	.071		
Do	1.4	6.9	8.3	8.8	18.4	.006	.063	.069	.071	Do.
Do	.7	8.4	9.1			.004	.069	.073		
Muscovite	6.0	22.0	28.0			.041	.154	.195		
Do	4.8	20.4	25.2	27.3	57.2	.039	.143	.182	.192	.119
Do	5.1	23.7	28.8			.040	.160	.200		
2 muscovite	5.0	20.0	25.0			.046	.140	.186		
Do	4.4	24.0	28.4	27.1	56.8	.044	.158	.202	.197	.124
Do	5.2	22.6	27.8			.048	.156	.204		
2 muscovite plus calcium carbonate	6.3	21.6	27.9			.050	.153	.203		
Do	5.2	22.4	27.6	26.8	56.1	.040	.146	.186	.193	.120
Do	4.9	20.8	25.7			.052	.140	.192		
2 muscovite plus 2 calcium carbonate	4.8	20.8	25.6			.043	.140	.183		
Do	6.1	22.4	28.5	27.5	57.6	.052	.156	.208	.198	.125
Do	5.8	22.6	28.4			.050	.154	.204		

TABLE VIII.—*Weight of rye crop and potash removed from soil*—Continued

Treatment.	Dry matter (grams).					Potash removed (grams).				
	Grain.	Straw.	Total.	Average total.	Relative rank of average.	Grain.	Straw.	Total.	Average total.	Average gain over no potash.
Orthoclase	3.0	15.6	18.6			0.022	0.106	0.128		
Do	3.8	15.0	18.8	18.5	38.7	.024	.100	.124	0.126	0.053
Do	3.1	15.2	18.3			.025	.102	.127		
2 orthoclase	2.8	14.6	17.4			.020	.100	.120		
Do	2.8	13.8	16.6	17.8	37.3	.020	.090	.110	.122	.049
Do	3.6	15.8	19.4			.026	.110	.136		
2 orthoclase plus calcium carbonate	3.6	16.0	19.6			.024	.102	.126		
Do	4.0	17.0	21.0	19.5	40.8	.029	.103	.132	.126	.053
Do	2.1	15.8	17.9			.020	.100	.120		
2 orthoclase plus 2 calcium carbonate	3.00	16.6	19.6			.025	.104	.129		
Do	2.6	16.4	19.0	19.0	39.8	.019	.104	.123	.126	.053
Do	2.4	16.0	18.4			.023	.103	.126		

Rye seems to remove more potash from the micas than from the feldspars. Microcline gives practically the same yield as when no potash is added. Orthoclase has given slightly greater yields, but nothing like so great as those of biotite and muscovite (Pl. 49, C).

In figure 3 will be found the graphical representation of rye produced with double applications of potash and lime.

As with the crop of oats, lime has produced very slight, if any, effect on liberating potash from the insoluble forms. Neither has crop growth nor the amount of potash removed by the crop been increased by its use

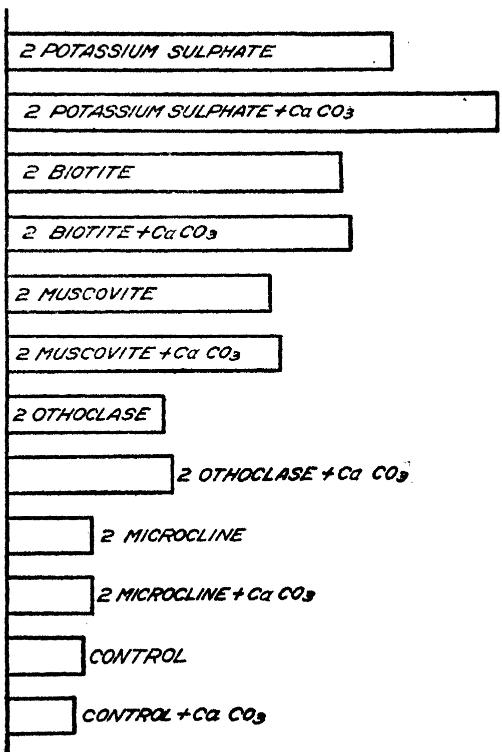

FIG. 3.—Rate of growth of rye under similar conditions fertilized with double applications of potash minerals and calcium carbonate.

Cowpea Crop

The rye roots were ground and mixed with the soil as with the preceding crops, and pots seeded with 15 seeds of Manetta variety of cowpeas on June 7, 1917. After germination, plantlets were reduced to six per pot. The crop was harvested on the following September 17, before the cowpeas had matured. This was done, as the plants had been injured by mildew.

In Table IX will be found the results of this experiment.

TABLE IX.—*Weight of cowpea crop and potash removed from soil*

Treatment.	Dry matter (grams).			Potash removed (grams).		
	Whole plant.	Average total.	Relative rank of average.	Whole plant.	Average total.	Average gain over no potash.
Potassium sulphate	30.4			0.374		
Do	27.0	27.8	63.3	.350	0.356	0.272
Do	26.1			.346		
2 potassium sulphate	31.0			.465		
Do	33.0	31.5	71.7	.476	.469	.385
Do	30.6			.468		
2 potassium sulphate plus calcium carbonate	40.2			.643		
Do	44.6	43.8	99.7	.669	.661	.577
Do	46.7			.672		
2 potassium sulphate plus 2 calcium carbonate	48.0			.720		
Do	42.0	43.9	100.0	.694	.698	.612
Do	41.6			.680		
Biotite	20.0			.300		
Do	17.6	19.1	43.5	.326	.313	.229
Do	19.7			.314		
2 biotite	21.4			.306		
Do	20.6	20.3	46.2	.312	.306	.222
Do	18.3			.300		
2 biotite plus calcium carbonate	24.0			.374		
Do	26.0	24.9	56.7	.386	.376	.292
Do	24.8			.370		
2 biotite plus 2 calcium carbonate	23.0			.302		
Do	22.0	24.0	54.6	.294	.303	.219
Do	27.0			.312		
Muscovite	14.0			.224		
Do	16.8	15.0	34.1	.236	.228	.144
Do	14.3			.224		
2 muscovite	15.0			.270		
Do	15.0	14.3	32.5	.264	.263	.179
Do	13.0			.256		
2 muscovite plus calcium carbonate	20.0			.302		
Do	22.6	21.5	48.9	.310	.310	.226
Do	22.0			.318		
2 muscovite plus 2 calcium carbonate	25.0			.326		
Do	23.0	24.1	54.8	.316	.318	.234
Do	24.2			.314		
Orthoclase	10.0			.150		
Do	8.4	8.1	18.4	.126	.138	.054
Do	8.0			.130		

TABLE IX.—*Weight of cowpea crop and potash removed from soil*—Continued

Treatment.	Dry matter (grams). Whole plant.	Dry matter (grams). Average total.	Dry matter (grams). Relative rank of average.	Potash removed (grams). Whole plant.	Potash removed (grams). Average total.	Potash removed (grams). Average gain over no potash.
2 othoclase	9.6	10.1	23.0	0.153	0.152	0.068
Do	11.2			.160		
Do	9.4			.142		
2 orthclase plus calcium carbonate	14.6	16.1	36.4	.202	.195	.111
Do	16.6			.174		
Do	17.8			.210		
2 orthoclase plus 2 calcium carbonate	15.0	16.3	36.5	.222	.201	.117
Do	16.0			.197		
Do	17.9			.184		
Microcline	4.8	5.5	12.4	.081	.088	.004
Do	5.6			.091		
Do	6.2			.094		
2 microcline	4.0	4.6	10.5	.068	.078	None.
Do	4.0			.084		
Do	5.9			.082		
2 microcline plus calcium carbonate	9.4	9.4	21.4	.108	.105	.021
Do	10.2			.110		
Do	8.6			.096		
2 microcline plus 2 calcium carbonate	10.0	9.6	21.8	.113	.106	.022
Do	10.2			.114		
Do	8.6			.092		
Control (no potash)	5.0	4.6	10.5	.090	.084	......
Do	4.8			.084		
Do	4.0			.078		
Control (no potash) plus calcium carbonate	10.0	8.5	19.3	.108	.105	.021
Do	7.2			.102		
Do	8.3			.105		
Control (no potash) plus 2 calcium carbonate	8.3	8.3	18.6	.101	.100	.016
Do	9.6			.103		
Do	7.2			.096		

Though the results are not as pronounced from cowpeas as with soybeans, the same general effect is produced Biotite and muscovite lead the insoluble minerals as carriers of potash. Orthoclase still seems to show a slight lead over microcline and where potash-carrying minerals were applied.

Lime has produced large increases where soluble potash is added, but its effect is not so great with the micaceous material as when soybeans were grown. This would indicate that, through the forces of weathering, a protective covering had formed around the particles of mica, preventing the plant roots from extracting as much potash as was done when the preceding legume was grown.

The rates of growth of cowpeas of the double applications of potash and lime are graphically given in figure 4.

Exceedingly small amounts of potash have been removed from the soil with the microline treatment. It gradually increases through treatments of orthoclase, muscovite, biotite until the maximum is reached with the soluble material (Pl. 49, D).

ACTIVE SOIL POTASH AFTER TWO YEARS' CROPPING

In order that the active or readily soluble soil potash left after two years' cropping might be determined, samples of each pot were subjected to solution in $N/5$ nitric acid. The samples were obtained by making three borings in each pot through the entire column of soil. The portions removed by each boring were well mixed, oven-dried, and bottled for analysis. Thirty-gm. portions were agitated with 500 cc. of $N/5$ nitric acid for 96 hours, after which the potash was determined colometrically. Table X gives the average amounts of potash removed by $N/5$ nitric acid.

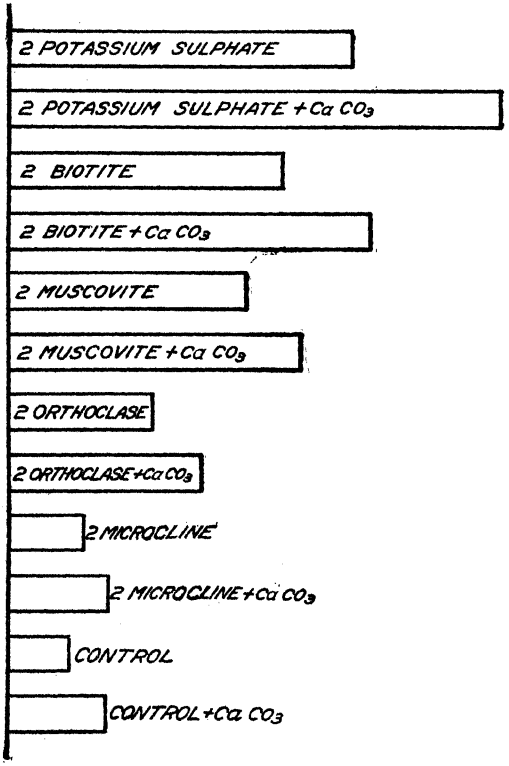

FIG. 4.—Rate of growth of cowpeas under similar conditions fertilized with double applications of potash minerals and calcium carbonate.

Considerably more potash was extracted by dilute acid when potassium sulphate had been added than when none had been used. Biotite and muscovite produced about the same quantity, more than double the amount of the controls. Very little, if any, increases could be discerned from the pots to which orthoclase or microcline had been applied.

Carbonate of lime does not have the slightest effect on any minerals used toward increasing the amount of potash soluble in $N/5$ nitric acid. In a majority of cases when the carbonate of lime has been used there are slight losses of potash from solution.

TABLE X.—*Active potash of soil after two years' cropping*

Treatment.	Potash carriers applied per pot.	Calcium carbonate applied per pot.	Potash recovered (p. p. m. of potassium oxid).	Gain or loss due to calcium carbonate.
	Gm.	*Gm.*		
Potassium sulphate	3. 57		34. 7	
2 potassium sulphate	7. 14		55. 9	
2 potassium sulphate plus calcium carbonate	7. 14	16. 71	53. 2	−2. 7
2 potassium sulphate plus 2 calcium carbonate	7. 14	33. 42	57. 6	+1. 7
Biotite	21. 49		24. 5	
2 biotite	42. 98		32. 6	
2 biotite plus calcium carbonate	42. 98	16. 71	26. 6	−6. 0
2 biotite plus 2 calcium carbonate	42. 98	33. 42	31. 8	−0. 8
Muscovite	19. 87		21. 8	
2 muscovite	39. 74		35. 9	
2 muscovite plus calcium carbonate	39. 74	16. 71	33. 4	−2. 5
2 muscovite plus 2 calcium carbonate	39. 74	33. 42	28. 6	−7. 3
Orthoclase	13. 55		13. 2	
2 orthoclase	27. 01		15. 2	
2 orthoclase plus calcium carbonate	27. 01	16. 71	12. 5	−2. 7
2 orthoclase plus 2 calcium carbonate	27. 01	33. 42	14. 6	−0. 6
Microcline	12. 55		13. 0	
2 microcline	25. 10		14. 8	
2 microcline plus calcium carbonate	25. 10	16. 71	10. 8	−4. 0
2 microcline plus 2 calcium carbonate	25. 10	33. 42	16. 0	+1. 2
Control (no potash)			12. 9	
Control (no potash) plus calcium carbonate		16. 71	14. 2	+1. 3
Control (no potash) plus 2 calcium carbonate		33. 42	10. 8	−3. 4

SUMMARY

The chief points brought out by this investigation are as follows:

(1) Little difference in the solubility of potash in water is found among the common soil-forming minerals: Biotite, muscovite, orthoclase, and microcline.

(2) Biotite and muscovite give up considerably more of their potash to solutions of carbonic acid than do orthoclase or microcline. The order in which potash is removed by this solvent is biotite, muscovite, orthoclase, and microcline.

(3) Lime as calcium bicarbonate does not increase the solubility of potash in any of the above minerals.

(4) Pot experiments which include the growth of four crops—oats, soybean, rye, and cowpea—that have had potash supplied in the form of minerals show that these plants can extract different amounts of this element from them. Biotite is able to produce four times the amount of dry matter of oats as microcline and 66 per cent as much as potassium sulphate. Muscovite produces nearly twice as much dry matter as orthoclase. The same general effect is caused from these carriers of potash with rye.

(5) Lime in the form of precipitated carbonate has not materially increased the dry matter or the potash removed from the soil by oats or

rye. The dry matter of soybean has been increased about 33 per cent when lime was used in conjunction with biotite. There was also a noticeably increased growth from muscovite caused by calcium carbonate. A much smaller increase was found from this material when the potash was applied as orthoclase or microcline.

(6) Lime caused the soybeans to remove more potash from the soil with potassium-sulphate, biotite, and muscovite treatments. This should not be taken necessarily to indicate that potash has been driven into solution, but that more favorable conditions for plant growth have been set up in the soil. More vigorous plants are thus produced, plants capable of removing more of this nutrient material. The results from the cowpeas were similar to those of soybeans.

(7) Slightly more potash was removed, after two years' cropping, by $N/5$ nitric acid from the pots fertilized with biotite and muscovite than from the control pots. No more potash was removed by this solvent where orthoclase and microcline had been added than from the controls.

(8) Lime does not appear to increase the solubility of the soil potash in $N/5$ nitric acid from any of the treatments.

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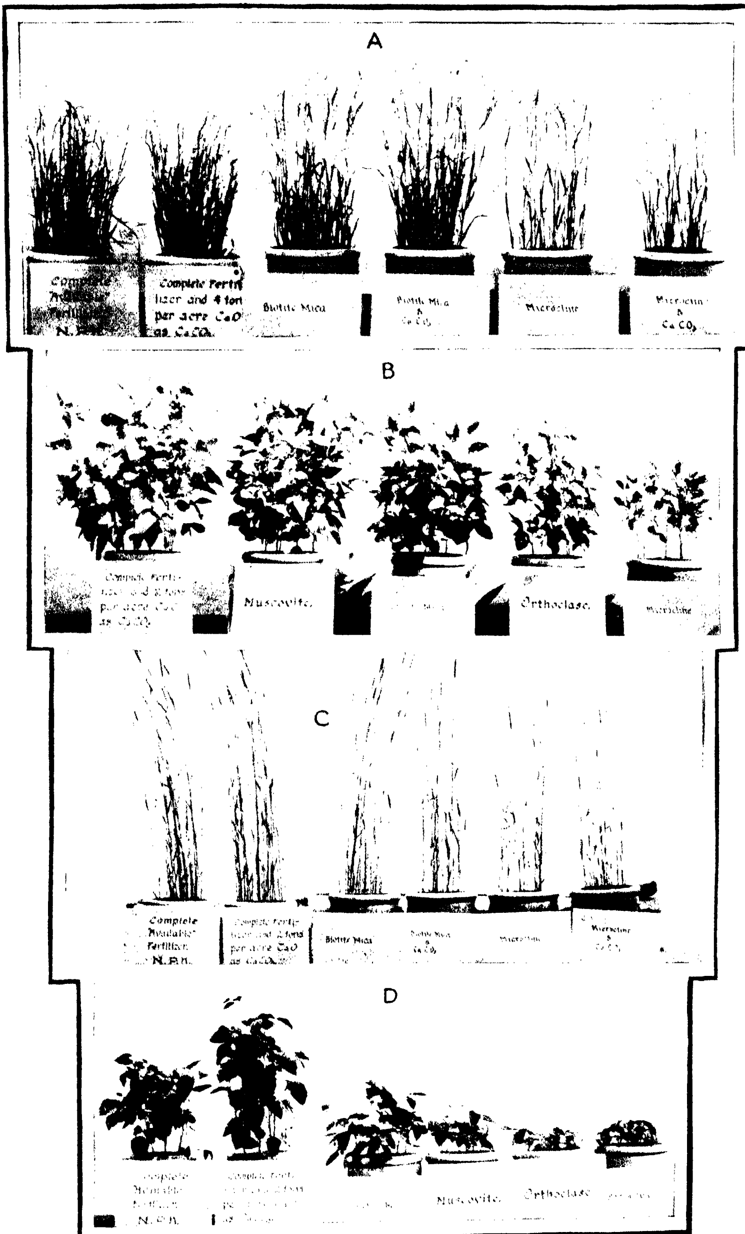
PLATE 49

A.—Oats, showing growth with potash from various minerals, with and without calcium carbonate.

B.—Soybeans, showing growth with potash from various minerals, with and without calcium carbonate.

C.—Rye, showing growth with potash from various minerals, with and without calcium carbonate.

D.—Cowpeas, showing growth with potash from various minerals, with and without calcium carbonate.



INFLUENCE OF REACTION ON NITROGEN-ASSIMILATING BACTERIA [1]

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INTRODUCTION

One of the most powerful factors influencing the growth of legumes is the reaction of the soil. Indeed, it has been known for a long time that alfalfa, clover, and related plants will not thrive on an acid soil. Some idea of the importance of this problem may be seen from the vast amount of literature dealing with this subject which has appeared in recent years. These reports are concerned largely with the nature of the acid constituents of the soil and with their influence on the growth of the higher plant. Details of these investigations are not essential here, since this paper presents the results obtained in a study of the influence of acidity on bacteria rather than on higher plants. Because of the intimate relation between host plant and bacteria in the case of legumes, it seems that the results of these tests may be useful in explaining the influence of soil acidity on legumes.

A number of scientists have noted in a general way the effect of total acidity or alkalinity on the nodule-forming bacteria and on their host plants. Their results are of interest, but they fail to give information in regard to the proper reaction for the growth of the bacteria without the host plant. Repeatedly the question is asked, How long will the legume bacteria live in soil in the absence of the legume? Undoubtedly the answer to this question involves a study of many factors, among which reaction is important. It is the purpose in this work to establish the relation of *Rhizobium leguminosarum* from different plants to acid and to alkali.

Before presenting the results obtained a brief review of some of the previous contributions to this subject will be made.

HISTORICAL REVIEW

More than 30 years ago Beijerinck (*1*) [2] in his study of *Rhizobium leguminosarum* noted that this organism is injured in a medium of *N/33.3* to *N/50* concentration of acid. He found that a medium prepared from a decoction of pea stems, reaction *N/166.6* malic acid, gave optimum conditions for growth.

From his observations Mazè (*17*) concluded that the legume bacteria may be divided into two great groups: (1) Those forms which are accus-

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[2] Reference is made by number (*italic*) to "Literature cited," p. 335-336.

tomed to acid soils and (2) those forms accustomed to alkaline soils. He claimed that the legume bacteria of the alkaline-soil group may be so modified, if cultivated on acid media, that the organism will become adapted to an acid reaction and produce nodules on the acid-resistant legumes.

Süchting's (*22*) results indicate that the legume bacteria retain their infecting power better in a neutral than in an acid medium. From the results of pot tests, Moore (*18*) reported that the legume bacteria would stand any degree of acidity or alkalinity of the soil that would permit the growth of its particular legume. This investigator found that legume bacteria flourished in media which contained as high as 0.05 per cent or *N/128.04* of free citric acid. According to Maassen and Müller (*16*), the legume bacteria are very sensitive to the reaction of the medium.

In connection with a study of the factors that influence nodule formation, Zipfel (*26, p. 127*) noted that the legume bacteria were not very sensitive to small amounts of acid or alkali. According to Hiltner (*13*), the sensitiveness of lupines to liming is a result of the injurious effect of the lime on the nodule bacteria. Whiting (*24*) claims that *Rhizobium leguminosarum* is not very sensitive to the reaction of the medium. He agrees with Moore that in the soil, legume bacteria and their host plant are equally resistant to acids and alkalis. Prucha (*20*) studied the influence of varying concentrations of hydrochloric acid and sodium hydroxid on *Rhizobium leguminosarum* of alfalfa. He found that no growth occurred on agar slants containing 10 per cent of normal hydrochloric acid or a concentration of *N/10*, and that toward sodium hydroxid the alfalfa organisms were much more resistant, about 30 per cent of normal alkali, a concentration of *N/3.3* being required to inhibit the growth of the bacteria. The results of his experiments indicate that large amounts of acid or alkali inhibit the growth of the legume organism and interfere with its power to infect.

Morgan and Gruzit (*19*) reported that an acid reaction of *N/1,200* was toxic to the growth of soil bacteria, while *N/1,000* alkali was approximately the most suitable reaction. In a later report Gruzit (*11*) found that the soil bacteria were very sensitive to an acid reaction. Sulphuric acid in a concentration of *N/1,200* killed about 99.6 per cent of the soil flora; of *N/1,400* about 93 per cent; and *N/2,840* inhibited the growth of 42 per cent of the bacteria. On the other hand, the maximum number of bacteria was noted in solutions with a reaction of *N/1,000* alkaline. The author concluded that the soil bacteria were more sensitive to acidity than were the corn-plant seedlings. A decrease in the number and activity of the denitrifying bacteria and of Azotobacter and of *Rhizobium leguminosarum* in acid soils has been noted by Loew (*15*).

From this brief review of the literature it will be seen that the results do not agree. An explanation for this variation may be found in the

method of determining reaction. More recent study has shown that bacterial processes are influenced by the hydrogen-ion concentration of the medium rather than by the total acidity. Therefore it was planned in this study to measure true acidity, concentration of hydrogen ions, as well as total acidity of the culture media.

INFLUENCE OF ACIDITY AND ALKALINITY ON THE GROWTH OF BACTERIA

The data reported deal with the effect of varying reactions on the multiplication of bacteria from some of the more important legumes, as well as on the multiplication of two different strains of Azotobacter. The term "strain" as used in this paper is applied to the same species of an organism isolated from different sources; for instance, the writers studied eight strains of the alfalfa organism, all of which were separated in pure culture from plants grown in widely separated sections and from soils of different reaction.

IDENTIFICATION OF LEGUME BACTERIA

To prove that the organisms employed were pure and true to name, all cultures were replated at least twice before their general characteristics were studied. Table I shows some of the cultural characteristics of these microorganisms. On standard nutrient-agar slopes growth is moderate, at first colorless, later a faint brown. In standard gelatin stab, growth is slow, chiefly at the top of the medium, brownish in color, and no liquefaction is noted for one or two weeks; however, in older cultures, three months, the gelatin is slowly liquefied. No gas is produced from dextrose, lactose, or saccharose broths, although the media become cloudy, and frequently a white membrane is formed which covers the surface. The organisms grow slowly in nitrate broth without gas production. In neutral litmus milk no change is noted during the first week; but at the end of the second or third week this medium becomes alkaline, and the dye is partly reduced. After two weeks bromcresol-purple milk inoculated with the legume bacteria becomes alkaline. The difference between the legume bacteria of different plants and different strains is perhaps best demonstrated by the rate of growth on mannitol-agar stroke cultures. On this medium the legume bacteria may be divided according to their amount of growth into three groups: scanty, moderate, and abundant growers. The organisms from different sources show decided variations in their growth in standard nutrient broth. The presence or absence of a membrane however, seems to bear no relation to the growth on mannitol-agar slopes. Except in the media already described, the legume bacteria exhibit close agreement in their cultural characteristics.

TABLE I.—*Cultural and biochemical characteristics of legume bacteria after two weeks at 28° C.*

Name of organism.	Mannitol agar, surface growth.	Standard nutrient broth.		Litmus milk.		Bromcresol-purple milk reaction.
		Surface growth.	Clouding.	Reaction.	Reduction.	
Alfalfa 1	Abundant.	None	Turbid	Alkaline	Slight at bottom	Alkaline.
Alfalfa 2	Scanty	do	do	do	No reduction	Do.
Alfalfa 3	Abundant.	Membranous.	do	do	do	Do.
Alfalfa 4	do	None	do	do	Slight at bottom	Do.
Alfalfa 5	do	Membranous.	do	do	do	Do.
Alfalfa 6	do	None	do	do	No reduction	Do.
Alfalfa 7	do	do	do	do	do	No change.
Alfalfa 8	Moderate	do	do			
Sweet clover 9	Abundant	do	do	Alkaline	Slight at bottom	Alkaline.
Garden pea 10	Moderate	do	do	do	No reduction	Do.
Field pea 11	do					
Vetch 12	do	None	Turbid	Alkaline	No reduction	Do.
Red clover 13	Abundant.	Membranous.	do	do	No reduction; slimy at top.	Do.
Red clover 14	do					
Common bean 15	Scanty					
Soy bean 16	do					
Soy bean 17	Abundant.	Membranous.	Turbid	Alkaline	No reduction; slimy at top.	Do.
Velvet bean 18	Moderate					
Lupine 19	Scanty	None		Alkaline	None	Do.
Lupine 20	Abundant	do	Turbid	do	Slight at bottom	Do.
Lupine 21	do	Membranous.	do	do	No reduction; slimy at top.	Do.
Lupine 22	do	do	do	do	do	Do.
Lupine 23	Scanty	None	do	do	No reduction	Do.

PRODUCTION OF NODULES

The final test of identity of a pure culture of the legume bacteria consisted in the formation of nodules on the legume from which the culture was obtained. For this purpose the leguminous plant and the microorganism were grown in large Pyrex tubes containing agar, under conditions which excluded all other forms of life. When nodules developed, a new isolation was made from the nodule and the organism secured in this way was compared with the original culture. In several cases these new cultures were again tested under sterile conditions for nodule formation. The lupines failed to grow well in the large test tubes, and for this legume a mixture of sterilized sand and soil was used. In every case the organism caused the formation of nodules, while the roots of the control plants were entirely free of nodules.

STAINING CHARACTERISTICS

The bacteria from the nodule or from agar slopes stain readily with carbol-fuchsin, gentian-violet, or methylene-blue. Perhaps the best preparations were obtained from the use of carbol-fuchsin, followed by a slight decolorization with dilute alcohol. The organism is Gram-negative when ethyl alcohol is used in the decolorizing process.

The number of flagella seems to depend on the source of the culture or on its age. This point, however, deserves more careful study. The fol-

lowing strains of alfalfa (*Medicago sativa*) and lupine (*Lupinus* sp.) were stained for flagella:

Alfalfa 1, 5, 6, 7, 8, peritrichous flagella.

Lupine 19, single, or rarely two, flagella.

The shape and general structure of the flagella of the lupine organism were different from those of the alfalfa organism. For instance, the flagella of lupine 19 are not so long and wavy as those of alfalfa.

EXPERIMENTAL PROCEDURE

It was realized from the beginning that the success of this study depended largely on the number of parallel tests and the number of different strains of bacteria employed. Therefore each experiment was repeated several times. In order to have comparable results all of the organisms were grown on the same medium, the composition of which is given below:

Mannitol ($C_6H_8(OH)_6$)	10.0 gm.
Magnesium sulphate ($MgSO_4+7H_2O$)	0.2 gm.
Dibasic potassium phosphate (K_2HPO_4)	0.2 gm.
Sodium chlorid (NaCl)	0.2 gm.
Calcium sulphate ($CaSO_4+2H_2O$)	0.1 gm.
Distilled water	1,000.0 cc.

Only the purest chemicals and conductivity water were used in preparing the culture medium. The reaction of the medium was usually neutral to phenolphthalein, although in some of the tests a very small amount of alkali was required to make it neutral. After dividing this culture solution among a series of flasks, the portions were sterilized and adjusted to different reactions with *N/10* or *N/20* acid and alkali. The normality of the culture medium is shown in the tabular data.

In beginning the experiments the acid and alkali limits of growth as determined by previous investigators were tried, and repeated tests were made until the critical point for the growth of the particular organism was reached.

Because of the importance of the acid-soil problem and the use of legumes in an acid system of agriculture, the greater part of this paper is concerned with the relation of legume bacteria to acidity, while their relation to alkalinity has received only limited study. With the exception of certain preliminary experiments, consideration was given not only to the total quantity of acid added but also to the effect of this acid on altering the hydrogen-ion concentration of the medium. Because of the low content of buffer substances in the mannitol medium, only small quantities of sulphuric acid were required to alter its hydrogen-ion concentration.

To determine the nature and extent of the buffer effect, preliminary tests of the hydrogen-ion concentration of the culture medium were made. For this purpose, a series of flasks of the medium was prepared in such

a way that one of its constituents was omitted from each test and the hydrogen-ion concentration measured immediately after the addition of the acid or alkali. It was stated in a previous paper that possibly the mannitol exerts a buffer effect. However, later investigations do not support this statement. The concentration of hydrogen ions in

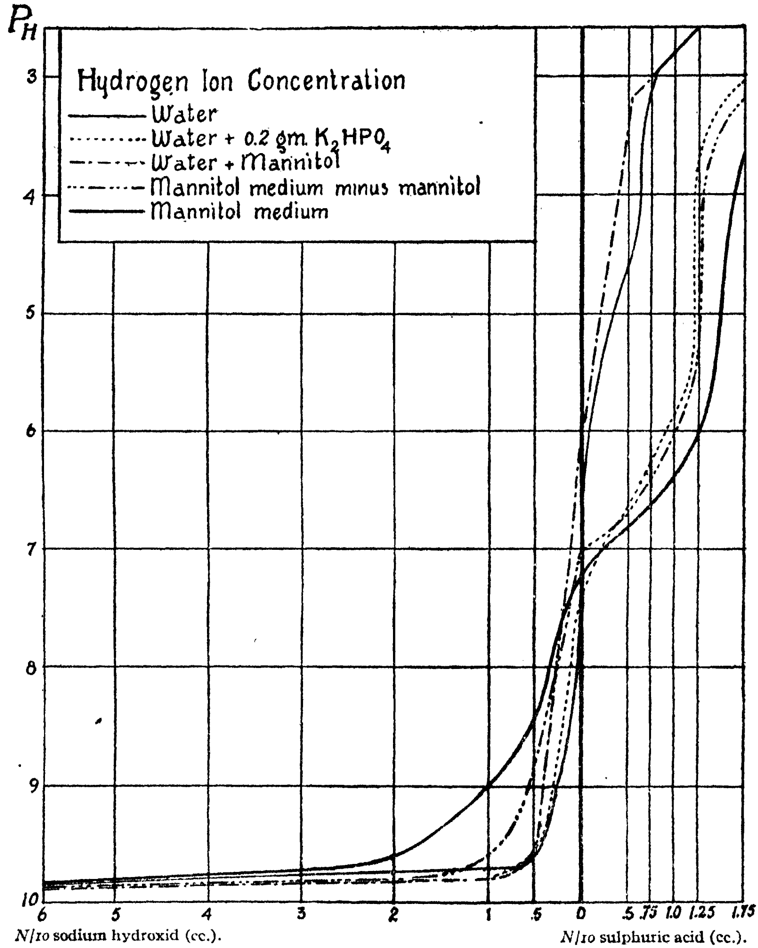

FIG. 1.—Graphs showing the buffer effect of the various constituents of mannitol medium.

pure water to which varying amounts of acid and alkali were added, as well as the concentration in the synthetic culture medium plus acid and alkali, is shown in figure 1. It appears from the graphs that the dibasic potassium phosphate is the chief buffer substance and that the mannitol has little effect on the concentration of hydrogen ions.

The concentration of hydrogen ions was measured by the colorimetric method as outlined by Clark and Lubs (*6*). The procedure was as follows: To a 10-cc. portion of the culture fluid the proper indicator was added, and the color developed was compared with the colors obtained on the addition of the same indicator to tubes of 10 cc. of the various "buffer solutions" of known hydrogen-ion concentration. Since the accuracy of this method depends on the standard "buffer solutions," these were prepared from chemicals purified as directed by Clark and Lubs, and the buffer mixtures checked by the electrometric method. In every case the two methods of measuring the hydrogen-ion exponent gave almost identical results. All of the data are reported as the hydrogen-ion exponent or P_H, instead of in terms of the normality of hydrogen ions. In the alkaline range, especially where large amounts of the base were used, the concentration of hydroxyl ions was frequently beyond the range of the indicators. Therefore the exponent of the hydrogen ion in the presence of large amounts of alkali is not correct.

INFLUENCE OF SULPHURIC ACID AND SODIUM HYDROXID ON THE REPRODUCTION OF NITROGEN-ASSIMILATING BACTERIA

TOTAL ACID AND ALKALI

A considerable number of experiments were made with *Rhizobium leguminosarum* from different plants and in general the agreement between these tests was good; therefore only a few of the typical ones are presented. The data show that the legume bacteria vary in their resistance to acidity, depending on the source of the organism. Taylor (*23*) has shown very clearly that acids, especially organic acids, vary in their degree of activity in checking the growth of bacteria, but that the inorganic acids, hydrochloric and nitric, however, show much similarity of action. In this work no attempt was made to try out different acids or alkalis, but rather to measure the action of sulphuric acid and of sodium hydroxid.

EXPERIMENTS WITH ALFALFA BACTERIA

In the following experiments a 1-cc. suspension of legume bacteria in sterilized water was used to inoculate 100 cc. of mannitol medium (p. 321) in 750-cc. Erlenmeyer flasks. At regular intervals of one week each the cultures were shaken vigorously and 1 cc. removed for plate counts. It was noticed early that increasing the acidity of a medium had a decided effect on the growth of the bacteria. Thus, the least acid members of a series were the first to show turbidity, while the more acid the reaction, the longer the period required for a noticeable turbidity to appear. The results obtained are given in Table II. In mannitol culture medium the injurious effect of alkali on legume bacteria is not noticeable unless added in amounts greater than *N/125*, while all growth is prevented in

N/62.5 sodium hydroxid. Toward gram-equivalent amounts of sulphuric acid these organisms behave differently; it seems that sulphuric acid is approximately 10 times as toxic to the bacteria as sodium hydroxid of the same normality. Growth is retarded in concentrations of *N/1,000*, and the cells are killed in solutions of the concentration of *N/500* sulphuric acid. These results do not agree with those of Beijerinck, who found a reaction of *N/166.6* acid gave optimum growth for *Rhizobium leguminosarum*. However, this difference in behavior of the bacteria no doubt is due to the difference in culture medium. From a glance at the data of this table it is plain that *R. leguminosarum* is much more sensitive to sulphuric acid than to gram-equivalent amounts of sodium hydroxid.

TABLE II.—*Effect of sulphuric acid and sodium hydroxid on the reproduction of alfalfa bacteria, strain 1*

No.	Normal acid or alkali in 100 cc. of medium	Concentration of acid or alkali.	Number of bacteria in 1 cc. of medium.					
			Inoculum.	After 1 week.	After 2 weeks.	After 3 weeks.	After 4 weeks.	After 6 weeks.
1	Neutral	Neutral	35,000	10,650,000	25,900,000	55,600,000	53,200,000	83,800,000
2	0.05 cc. sulphuric acid.	*N/2,000*	35,000	9,000,000	21,520,000	44,600,000	32,500,000	54,000,000
3	0.1 cc. sulphuric acid.	*N/1,000*	35,000	7,010,000	19,580,000	44,300,000	31,000,000	19,400,000
4	0.2 cc. sulphuric acid.	*N/500*	35,000	None.	None.	None.	None.	None.
5	0.3 cc. sulphuric acid.	*N/333*	35,000	None.	None.	None.	None.	None.
6	0.1 cc. sodium hydroxid.	*N/1,000*	35,000	7,000,000	20,280,000	42,900,000	29,200,000	65,500,000
7	0.2 cc. sodium hydroxid.	*N/500*	35,000	5,070,000	18,280,000	31,600,000	30,100,000	12,500,000
8	0.4 cc. sodium hydroxid.	*N/250*	35,000	3,750,000	13,220,000	23,600,000	29,800,000	28,500,000
9	0.8 cc. sodium hydroxid.	*N/125*	35,000	7,110,000	14,540,000	26,600,000	21,900,000	23,600,000
10	1.6 cc. sodium hydroxid.	*N/62.5*	35,000	None.	None.	None.	None.	None.

A comparison of the effect of treatment on the number of bacteria at various intervals of time failed to show any decided difference. In relation to time, the acid or alkali exerted approximately the same effect on the multiplication of bacteria after one or six weeks.

EXPERIMENTS WITH AZOTOBACTER, ALFALFA, LUPINE, RED CLOVER, AND SOYBEAN BACTERIA

The behavior of alfalfa bacteria is in accord with our knowledge of the host plant—that is, they are sensitive to acidity. The question which naturally suggests itself is that of the relation of other strains of legume bacteria to different reactions. In addition to the legume bacteria, one strain of Azotobacter was studied. Only one count, two weeks after inoculation, was made, since the number of organisms at different intervals of time had failed to show any marked variation. The averages of the plate counts after two weeks are given in Table III. The results with alfalfa confirm those of the earlier tests and show that this organism is

killed quickly in solutions containing small amounts of acid. The difference in behavior of the bacteria from alfalfa and lupine is evident. The latter are more resistant to sulphuric acid than the former.

TABLE III.—*Effect of sulphuric acid and sodium hydroxid on the reproduction of nitrogen-fixing bacteria*

No.	Normal acid or alkali in 100 cc. of medium.	Concentration of acid or alkali.	Number of bacteria in 1 cc. of medium two weeks after inoculation.				
			Alfalfa 1 (inoculum 350,000).	Lupine 21 (inoculum 1,570,000).	Red clover 13 (inoculum 1,300,000).	Soybean 17 (inoculum 1,100,000).	Azotobacter 131.
1	Neutral	Neutral	12,300,000	30,000,000	17,100,000	29,100,000	1,560,000
2	0.1 cc. sulphuric acid	*N*/1,000	7,200,000	21,900,000	Lost.	22,300,000	212,000
3	0.2 cc. sulphuric acid	*N*/500	None.	700	Lost.	None.	None.
4	0.5 cc. sulphuric acid	*N*/200	None.	None.	None.	None.	None.
5	0.1 cc. sodium hydroxid	*N*/1,000	11,900,000	24,300,000	15,600,000	21,900,000	7,120,000
6	0.2 cc. sodium hydroxid	*N*/500	9,800,000	26,100,000	17,800,000	22,300,000	3,820,000
7	0.5 cc. sodium hydroxid	*N*/200	Lost.	11,500,000	24,600,000	15,100,000	None.
8	1.0 cc. sodium hydroxid	*N*/100	8,600,000	5,900	12,600,000	7,100,000	None.

One very striking fact shown in the data of this experiment is the narrow limits of growth of Azotobacter. This organism is readily affected by small amounts of acid or alkali, the limits of growth are approximately *N*/1,000 acid and *N*/500 alkali. These data are in agreement with the results of previous investigators. For instance, it has been shown by Christensen and his associates (*3*, *4*, *5*) that the formation of Azotobacter film in mannitol cultures inoculated with soil is correlated with the reaction of the soil—that is, acid soils fail to show any film.

From the data of the previous tests no conclusions can be drawn with respect to the acid or alkali limit of growth of bacteria except within a relatively wide range. Therefore further tests were arranged in such a way as to give a series of cultures of varying concentration of acid and alkali. Here the difference between the reactions of the cultures was less than in former experiments. Instead of counting the total number of bacteria at different intervals, the cultures were incubated for 21 days and then tested for the presence or absence of living bacteria. The turbidity of the culture was noted, the presence of bacteria determined as shown by a stained mount, and mannitol-agar slants were inoculated with a loop of the cultures. The presence or absence of growth on the agar slants was taken as an indication of the presence or absence of living bacteria in the culture.

A comparison of the development of different legume bacteria in media of varying reactions is presented in Table IV. The data here reported were obtained from a series of separate tests. The greater resistance of the lupine bacteria to acidity as compared with the alfalfa bacteria is clearly shown by the results presented in this table. Seven different strains of the alfalfa organism and four of lupine were studied, and in each test these different strains of the alfalfa and lupine organism behaved alike. The acid range for alfalfa bacteria is approximately

N/909 and for lupine bacteria *N/588*. The resistance of the lupine bacteria to acids is in accord with the results of analyses of the root juices. Lemmermann (*14*) has shown that the extract from roots of lupines is more acid than that from roots of beans, peas, vetch, or serradella.

TABLE IV.—*Effect of sulphuric acid on the reproduction of legume bacteria after 21 days.*

No.	Normal sulphuric acid in 100 cc. of medium.	Concentration of acid.	Result after 21 days.						
			Alfalfa 1.	Alfalfa 2.	Alfalfa 3.	Alfalfa 4.	Alfalfa 5.	Alfalfa 7.	Alfalfa 8.
	Cc.								
1	Neutral	Neutral	Growth	Growth	Growth	Growth	Growth	Growth	Growth
2	0.025	*N/4,000*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
3	0.050	*N/2,000*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
4	0.072	*N/1,389*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
5	0.075	*N/1,333*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
6	0.080	*N/1,250*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
7	0.088	*N/1,136*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
8	0.092	*N/1,087*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
9	0.096	*N/1,042*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
10	0.100	*N/1,000*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
11	0.110	*N/909*	None...	None...	None...	None...	None...	None...	None.
12	0.120	*N/833*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
13	0.125	*N/800*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
14	0.130	*N/769*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
15	0.132	*N/757*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
16	0.135	*N/741*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
17	0.140	*N/714*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
18	0.145	*N/690*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
19	0.150	*N/667*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
20	0.156	*N/641*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
21	0.160	*N/625*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
22	0.168	*N/595*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
23	0.170	*N/588*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
24	0.180	*N/556*	...do...	...do...	...do...	...do...	...do...	...do...	Do.
25	0.190	*N/526*	...do...	...do...	...do...	...do...	...do...	...do...	Do.

No.	Normal sulphuric acid in 100 cc. of medium.	Concentration of acid.	Result after 21 days.					
			Sweet clover 9.	Garden pea 10.	Field pea 11.	Vetch 12.	Red clover. 13.	Red clover 14.
	Cc.							
1	Neutral	Neutral	Growth	Growth	Growth	Growth	Growth	Growth.
2	0.025	*N/4,000*	...do...	...do...	...do...	...do...	...do...	Do.
3	0.050	*N/2,000*	...do...	...do...	...do...	...do...	...do...	Do.
4	0.072	*N/1,389*	...do...	...do...	...do...	...do...	...do...	Do.
5	0.075	*N/1,333*	...do...	...do...	...do...	...do...	...do...	Do.
6	0.080	*N/1,250*	...do...	...do...	...do...	...do...	...do...	Do.
7	0.088	*N/1,136*	...do...	...do...	...do...	...do...	...do...	Do.
8	0.092	*N/1,087*	...do...	...do...	...do...	...do...	...do...	Do.
9	0.096	*N/1,042*	...do...	...do...	...do...	...do...	...do...	Do.
10	0.100	*N/1,000*	...do...	...do...	...do...	...do...	...do...	Do.
11	0.110	*N/909*	None...	None...	None...	None...	None...	None.
12	0.120	*N/833*	...do...	...do...	...do...	...do...	...do...	Do.
13	0.125	*N/800*	...do...	...do...	...do...	...do...	...do...	Do.
14	0.130	*N/769*	...do...	...do...	...do...	...do...	...do...	Do.
15	0.132	*N/757*	...do...	...do...	...do...	...do...	...do...	Do.
16	0.135	*N/741*	...do...	...do...	...do...	...do...	...do...	Do.
17	0.140	*N/714*	...do...	...do...	...do...	...do...	...do...	Do.
18	0.145	*N/690*	...do...	...do...	...do...	...do...	...do...	Do.
19	0.150	*N/667*	...do...	...do...	...do...	...do...	...do...	Do.
20	0.156	*N/641*	...do...	...do...	...do...	...do...	...do...	Do.
21	0.160	*N/625*	...do...	...do...	...do...	...do...	...do...	Do.
22	0.168	*N/595*	...do...	...do...	...do...	...do...	...do...	Do.
23	0.170	*N/588*	...do...	...do...	...do...	...do...	...do...	Do.
24	0.180	*N/556*	...do...	...do...	...do...	...do...	...do...	Do.
25	0.190	*N/526*	...do...	...do...	...do...	...do...	...do...	Do.

TABLE IV.—*Effect of sulphuric acid on the reproduction of legume bacteria after 21 days*—Continued

No.	Normal sulphuric acid in 100 cc. of medium.	Concentration of acid.	Result after 21 days.					
			Velvet bean 18.	Soy-bean 17.	Lupine 19.	Lupine 20.	Lupine 21.	Lupine 22.
	Cc.							
1	Neutral	Neutral	Growth	Growth	Growth	Growth	Growth	Growth.
2	0.025	*N/4,000*	do	do	do	do	do	Do.
3	0.050	*N/2,000*	do	do	do	do	do	Do.
4	0.072	*N/1,389*	do	do	do	do	do	Do.
5	0.075	*N/1,333*	do	do	do	do	do	Do.
6	0.080	*N/1,250*	do	do	do	do	do	Do.
7	0.088	*N/1,136*	do	do	do	do	do	Do.
8	0.092	*N/1,087*	do	do	do	do	do	Do.
9	0.096	*N/1,042*	do	do	do	do	do	Do.
10	0.100	*N/1,000*	do	do	do	do	do	Do.
11	0.110	*N/909*	do	do	do	do	do	Do.
12	0.120	*N/833*	do	do	do	do	do	Do.
13	0.125	*N/800*	do	do	do	do	do	Do.
14	0.130	*N/769*	do	do	do	do	do	Do.
15	0.132	*N/757*	do	do	do	do	do	Do.
16	0.135	*N/741*	do	do	do	do	do	Do.
17	0.140	*N/714*	do	do	do	do	do	Do.
18	0.145	*N/690*	do	do	do	do	do	Do.
19	0.150	*N/667*	do	do	do	do	do	Do.
20	0.156	*N/641*	do	do	do	do	do	Do.
21	0.160	*N/625*	None	None	do	do	do	Do.
22	0.168	*N/505*	do	do	do	do	do	Do.
23	0.170	*N/588*	do	do	None	None	do	Do.
24	0.180	*N/556*	do	do	do	do	None	None.
25	0.190	*N/526*	do	do	do	do	do	Do.

Because of the large number of cultures used and the small difference in amount of acid between each culture it is possible to separate the legume bacteria into classes, depending on their resistance to acidity. If grouped in this way, the alfalfa organism would stand at the alkaline end of the scale, the lupine organism at the acid end. Sweet clover, vetch, garden pea, red clover, velvet bean, and soybean organisms would occupy an intermediate position and about in the order named, graded from acid sensitive to acid resistant.

In the case of lupine and alfalfa, the different strains of the same organism show remarkable agreement and support the statement that the influence of acid on the lower plant, *Rhizobium leguminosarum*, is similar to the influence of acid on the higher plant, the legume.

EXPERIMENTS WITH AZOTOBACTER

The selection of Azotobacter was prompted by the fact that various investigators have reported that the growth of this organism may be used as an indicator of the reaction of soil. In order to test the influence of acid and alkali on Azotobacter, a series of mannitol cultures was prepared and treated as given in the preceding experiments. Three separate tests were made and the data recorded in Table V. In every culture in which acid or alkali was used a very marked effect on growth was noted. When compared with the legume organism regardless of the source, it is plain that Azotobacter is much more sensitive to changes in reaction.

Apparently the acid limit for Azotobacter is about *N/1,333.3* and the alkaline limit of growth about *N/1,000*. In relation to nitrates, Azotobacter behaves in a somewhat similar manner—that is, it is more sensitive to high concentrations than is *Rhizobium leguminosarum* (*12, p. 209*).

TABLE V.—*Effect of sulphuric acid and sodium hydroxid on the reproduction of Azotobacter*

No.	Normal acid or alkali in 100 cc. of medium.	Concentration of acid or alkali.	Result after 21 days.		
			Azotobacter. 130.	Azotobacter 131.	Azotobacter 130.
1	Neutral		Growth.	Growth.	Growth.
2	0. 025 cc. sulphuric acid	*N/4,000*	...do...	...do...	Do.
3	0. 050 cc. sulphuric acid	*N/2,000*	...do...	...do...	Do.
4	0. 075 cc. sulphuric acid	*N/1,333*	...do...	...do...	Trace.
5	0. 100 cc. sulphuric acid	*N/1,000*	None...	None...	None.
6	0. 110 cc. sulphuric acid	*N/909*	...do...	...do...	Do.
7	0. 120 cc. sulphuric acid	*N/833*	...do...	...do...	Do.
8	0. 125 cc. sulphuric acid	*N/800*	...do...	...do...	Do.
9	0. 050 cc. sodium hydroxid	*N/2,000*	Growth.	Growth.	
10	0. 100 cc. sodium hydroxid	*N/1,000*	...do...	...do...	
11	0. 200 cc. sodium hydroxid	*N/500*	None...	Trace...	
12	0. 500 cc. sodium hydroxid	*N/200*	...do...	None...	

INFLUENCE OF SULPHURIC ACID AND SODIUM HYDROXID ON THE REPRODUCTION OF NITROGEN-ASSIMILATING BACTERIA

DISSOCIATED ACID OR ALKALI

The marked influence of reaction upon the nitrogen-fixing bacteria, especially certain strains of the legume organisms and Azotobacter, has been pointed out in the results of this paper. The evidence submitted is sufficient to warrant the conclusion that sulphuric acid in mannitol solution is more toxic than is the hydrogen equivalent amount of sodium hydroxid. This difference in the action of acid and alkali may be due in part to their difference in dissociation. Attention was called to this point in an earlier paper (*10*).

EXPERIMENTS WITH ALFALFA AND LUPINE BACTERIA

In Table VI are given the hydrogen-ion exponents for each of 16 culture solutions and the growth of the organisms as shown by transfers to agar slopes. The cultures are arranged in order of decreasing acidity and the reaction of the culture medium varies as shown in the table from P_H 4.6 to P_H 9.8; the readings for the high alkaline range are not absolute. A study of the data shows that the alfalfa bacteria are more sensitive to true acidity than are the lupine bacteria. The acid limit of growth as shown in this test is between P_H 5.4 and P_H 6.0 for alfalfa and lower than P_H 4.6 for lupine. In all cases there was a good agreement between the growth of the different strains of the same organism.

TABLE VI.—*Effect of the concentration of hydrogen ions on the reproduction of alfalfa and lupine bacteria*

No.	P_H.	Result after 21 days.					
		Alfalfa 6.	Alfalfa 3.	Alfalfa 5.	Lupine 19.	Lupine 19.	Lupine 21.
1	4.6		None	None		Growth	Growth.
2	5.4	None			Growth		
3	6.0	Growth	Growth	Growth	do	Growth	Do.
4	6.2	do			do		
5	6.4		Growth	Growth		Growth	Do.
6	6.6	Growth	do	do	Growth	do	Do.
7	6.8	do	do	do	do	do	Do.
8	7.4	do	do	do	do	do	Do.
9	8.4	do	do	do	do	do	Do.
10	8.6	do			do		
11	8.8	do			do		
12	9.0		Growth	Growth	do	Growth	Do.
13	9.2	Growth			None		
14	9.4	do			do		
15	9.6		Growth	Growth		Growth	Do.
16	9.8		do	None	None	None	None.

A second experiment was set up similar to the preceding except that only the acid range was tested. Twenty-one strains of legume bacteria were studied. All the data for this test are summarized in Table VII. An examination of the results shows clearly that the growth of the legume bacteria in culture solutions of varying reactions is proportional to the hydrogen-ion concentration of the medium, and it is probable that their difference in resistance to hydrogen ions is related to the reaction of the sap of the host plant. As shown in the data of Tables VI and VII, the organisms of alfalfa are the most sensitive of the legume bacteria to the concentration of hydrogen ions, while the lupine bacteria are the most resistant. In relation to true acidity, the sweet-clover, garden-pea, field-pea, vetch, common-bean, red-clover, soybean, and velvet-bean organisms occupy a position between alfalfa and lupine bacteria. The velvet-bean and the soybean organisms show considerable resistance to an increase in hydrogen-ion concentration.

It is of interest to note the results obtained by other investigators. Brünn (2) found that *Bacillus coli* is killed within 24 hours if exposed to an acid reaction of $P_H=4.7$, but not of $P_H=5.0$. Wolf and Harris (25) reported that the difference between the reaction which just permits growth and the reaction which prevents growth is not great. They suggest the term "critical P_H" which is obtained by taking the average of the two values, the P_H which just permits growth and the P_H which inhibits growth. They found that the critical reaction for *Bacillus welchii* (*B. perfringens*) is about $P_H=4.82$ and for *B. sporogenes* (Metchnikoff) about $P_H=4.94$. In both tests glucose peptone media were used.

TABLE VII.—*Effect of concentration of hydrogen ions on the reproduction of legume bacteria after 21 days*

No.	P_H.	Result after 21 days.						
		Alfalfa 1.	Alfalfa 2.	Alfalfa 3.	Alfalfa 4.	Alfalfa 5.	Alfalfa 7.	Alfalfa 8.
1	3.0	None	None	None	None	None	None	None.
2	3.1	do	do	do	do	do	do	Do.
3	3.2	do	do	do	do	do	do	Do.
4	3.4	do	do	do	do	do	do	Do.
5	3.5	do	do	do	do	do	do	Do.
6	3.6	do	do	do	do	do	do	Do.
7	3.7	do	do	do	do	do	do	Do.
8	3.8	do	do	do	do	do	do	Do.
9	4.0	do	do	do	do	do	do	Do.
10	4.1	do	do	do	do	do	do	Do.
11	4.3	do	do	do	do	do	do	Do.
12	4.6	do	do	do	do	do	do	Do.
13	4.8	do	do	do	do	do	do	Do.
14	5.0	Growth	Growth	Growth	Growth	Growth	Growth	Growth.
15	5.2	do	do	do	do	do	do	Do.
16	5.4	do	do	do	do	do	do	Do.
17	5.5	do	do	do	do	do	do	Do.
18	5.6	do	do	do	do	do	do	Do.
19	5.7	do	do	do	do	do	do	Do.
20	5.9	do	do	do	do	do	do	Do.
21	6.1	do	do	do	do	do	do	Do.
22	6.2	do	do	do	do	do	do	Do.
23	6.3	do	do	do	do	do	do	Do.
24	6.4	do	do	do	do	do	do	Do.
25	6.6	do	do	do	do	do	do	Do.
26	6.8	do	do	do	do	do	do	Do.
27	7.0	do	do	do	do	do	do	Do.
28	7.1	do	do	do	do	do	do	Do.

No.	P_H.	Result after 21 days.						
		Sweet clover 9.	Garden pea 10.	Field pea 11.	Vetch 12.	Red clover 14.	Red clover 13.	Bean 15.
1	3.0	None	None	None	None	None	None	None.
2	3.1	do	do	do	do	do	do	Do.
3	3.2	do	do	do	do	do	do	Do.
4	3.4	do	do	do	do	do	do	Do.
5	3.5	do	do	do	do	do	do	Do.
6	3.6	do	do	do	do	do	do	Do.
7	3.7	do	do	do	do	do	do	Do.
8	3.8	do	do	do	do	do	do	Do.
9	4.0	do	do	do	do	do	do	Do.
10	4.1	do	do	do	do	do	do	Do.
11	4.3	do	do	do	do	Growth	Growth	Growth.
12	4.6	do	do	do	do	do	do	Do.
13	4.8	Growth	Growth	Growth	Growth	do	do	Do.
14	5.0	do	do	do	do	do	do	Do.
15	5.2	do	do	do	do	do	do	Do.
16	5.4	do	do	do	do	do	do	Do.
17	5.5	do	do	do	do	do	do	Do.
18	5.6	do	do	do	do	do	do	Do.
19	5.7	do	do	do	do	do	do	Do.
20	5.9	do	do	do	do	do	do	Do.
21	6.1	do	do	do	do	do	do	Do.
22	6.2	do	do	do	do	do	do	Do.
23	6.3	do	do	do	do	do	do	Do.
24	6.4	do	do	do	do	do	do	Do.
25	6.6	do	do	do	do	do	do	Do.
26	6.8	do	do	do	do	do	do	Do.
27	7.0	do	do	do	do	do	do	Do.
28	7.1	do	do	do	do	do	do	Do.

TABLE VII.—*Effect of concentration of hydrogen ions on the reproduction of legume bacteria after 21 days*—Continued

No.	P_H.	Result after 21 days.						
		Soybean 16.	Soybean 17.	Velvet bean 18.	Lupine 19.	Lupine 20.	Lupine 21.	Lupine 22.
1	3.0	None.	None.	None.	None.	None.	None.	None.
2	3.1	do.	do.	do.	do.	do.	do.	Do.
3	3.2	do.	do.	do.	Growth.	Growth.	Growth.	Growth.
4	3.4	do.	Growth.	Growth.	do.	do.	do.	Do.
5	3.5	Growth.	do.	do.	do.	do.	do.	Do.
6	3.6	do.	do.	do.	do.	do.	do.	Do.
7	3.7	do.	do.	do.	do.	do.	do.	Do.
8	3.8	do.	do.	do.	do.	do.	do.	Do.
9	4.0	do.	do.	do.	do.	do.	do.	Do.
10	4.1	do.	do.	do.	do.	do.	do.	Do.
11	4.3	do.	do.	do.	do.	do.	do.	Do.
12	4.6	do.	do.	do.	do.	do.	do.	Do.
13	4.8	do.	do.	do.	do.	do.	do.	Do.
14	5.0	do.	do.	do.	do.	do.	do.	Do.
15	5.2	do.	do.	do.	do.	do.	do.	Do.
16	5.4	do.	do.	do.	do.	do.	do.	Do.
17	5.5	do.	do.	do.	do.	do.	do.	Do.
18	5.6	do.	do.	do.	do.	do.	do.	Do.
19	5.7	do.	do.	do.	do.	do.	do.	Do.
20	5.9	do.	do.	do.	do.	do.	do.	Do.
21	6.1	do.	do.	do.	do.	do.	do.	Do.
22	6.2	do.	do.	do.	do.	do.	do.	Do.
23	6.3	do.	do.	do.	do.	do.	do.	Do.
24	6.4	do.	do.	do.	do.	do.	do.	Do.
25	6.6	do.	do.	do.	do.	do.	do.	Do.
26	6.8	do.	do.	do.	do.	do.	do.	Do.
27	7.0	do.	do.	do.	do.	do.	do.	Do.
28	7.1	do.	do.	do.	do.	do.	do.	Do.

The limit of growth and critical P_H values for the legume bacteria and Azotobacter are about as follows:

No.	Organism.	Acid value of P_H which allows growth.	Acid value of P_H which inhibits growth.	Mean value of P_H or the critical P_H.
1	*Rhizobium leguminosarum* from alfalfa	5.0	4.8	4.9
2	*Rhizobium leguminosarum* from sweet clover	5.0	4.8	4.9
3	*Rhizobium legunimosarum* from garden pea	4.8	4.6	4.7
4	*Rhizobium leguminosarum* from field pea	4.8	4.6	4.7
5	*Rhizobium leguminosarum* from vetch	4.8	4.6	4.7
6	*Rhizobium leguminosarum* from red clover	4.3	4.1	4.2
7	*Rhizobium leguminosarum* from bean	4.3	4.1	4.2
8	*Rhizobium leguminosarum* from soybean	3.4	3.2	3.3
9	*Rhizobium leguminosarum* from velvet bean	3.4	3.2	3.3
10	*Rhizobium leguminosarum* from lupine	3.2	3.1	3.15
11	Azotobacter	6.6	6.4	6.5

If the critical P_H value of the legume bacteria be compared with the growth of the leguminous plant in soil of varying reaction, it will be noted that the bacteria in relation to acidity behave similar to their host plants. Here, then, is a characteristic of the legume bacteria which separates these organisms into different groups, acid sensitive, acid resistant, and no doubt a long list of organisms intermediate between the two extremes.

EXPERIMENTS WITH AZOTOBACTER

The general plan followed was similar to that outlined in Table V. The results obtained are given in Table VIII. Here, again, the extreme sensitiveness of Azotobacter to true acid or alkali is plainly shown. The limit of hydrogen-ion concentration for the growth of this organism is about P_H 6.5. In agreement with the results of the previous experiments it is clear that toward hydrogen ions Azotobacter is more sensitive than any of the legume bacteria used in this investigation. The narrow limits of growth for Azotobacter, P_H 6.6 to 8.4 or 8.8, indicate that the growth of this organism may be used to measure the reaction of various substances.

TABLE VIII.—*Effect of the concentration of hydrogen ions on the reproduction of Azotobacter*

No.	P_H.	Azotobacter 130.	Azotobacter 131.	Azotobacter 130.
1	4.6		None	None.
2	5.4	None	do	Do.
3	6.0	do	do	Do.
4	6.2	do		Do.
5	6.4		None	Do.
6	6.6	None	Growth	Growth.
7	6.8	Growth	do	Do.
8	7.4	do	do	Do.
9	8.4	do	do	
10	8.6	None		
11	8.8	do	Growth	
12	9.0	do		
13	9.2			
14	9.4	None		
15	9.6		None	
16	9.8		do	

TABLE IX.—*Effect of Rhizobium leguminosarum and of Azotobacter on the reaction of the culture medium*

Name of organism.	P_H value.			Name of organism.	P_H value.		
	Beginning.	End.	Difference.		Beginning.	End.	Difference.
Alfalfa 1	7.2	7.1	0.1	Garden bean 15	7.2	6.9	0.3
Alfalfa 2	7.2	7.0	.2	Cowpea 28	7.2	7.1	.1
Alfalfa 3	7.2	7.0	.2	Vetch 12	7.2	7.0	.2
Alfalfa 4	7.2	7.0	.2	Field pea 11	7.2	7.0	.2
Alfalfa 7	7.2	6.9	.3	Garden pea 24	7.2	6.9	.3
Alfalfa 8	7.2	7.0	.2	Garden pea 25	7.2	7.0	.2
Sweet clover 9	7.2	7.0	.2	Garden pea 26	7.2	6.8	.4
Sweet clover 29	7.2	7.0	.2	Serradella 27	7.2	7.1	.1
Red clover 13	7.2	7.0	.2	Lupine 19	7.2	7.2	.0
Red clover 14	7.2	6.8	.4	Lupine 20	7.2	7.1	.1
Soybean 16	7.2	7.1	.1	Lupine 22	7.2	7.0	.2
Soybean 17	7.2	7.0	.2	Lupine 21	7.2	7.1	.1
Velvet bean 18	7.2	7.2	.0	Azotobacter 131	7.2	5.1	2.1

INFLUENCE OF NITROGEN-ASSIMILATING BACTERIA ON THE REACTION OF THE MEDIUM

Since the legume bacteria show a difference in behavior toward reaction of the culture medium, it was thought that the growth of the different strains might cause a noticeable variation in the reaction of the medium. Accordingly, the reaction was measured by titrating the cultures with *N/20* acid or alkali at the time of inoculation and again four weeks later. The results of titrations failed to show any decided change in the reaction of the culture medium after the growth of the different organisms, although there was a slight increase in acidity. Similar results were reported in an earlier publication (9).

The results of hydrogen-ion measurements of the inoculated and uninoculated culture solutions showed a small but distinct increase in acidity. In this test saccharose solution was used in place of the mannitol. In Table IX only the averages of duplicate cultures are given. As a rule, the change in the reaction due to the growth of *R. leguminosarum* in the saccharose solution was from P_H 0.1 to 0.4, the average about P_H 0.2. This gain in acidity is very small when compared with that produced by Azotobacter—namely, 2.1. Because of the turbidity of the culture medium, which is caused by the great number of bacteria, it seems strange that there is only a slight change in the hydrogen-ion concentration. Determinations of the amount of sugar consumed by these organisms in liquid media offer an explanation for the small increase in acid. It has been found that *R. leguminosarum* may develop in enormous numbers without consuming more than 4 to 5 per cent of the total amount of sugar in the medium (9).

SUMMARY

The behavior of the legume bacteria as well as Azotobacter toward small amounts of acid or alkali depends upon many factors: Chief among these are the nature of the medium and the dissociation of the acid and alkali.

All the results point to the fact that *R. leguminosarum* regardless of strain, does not persist for any length of time in a medium, the reaction of which prevents reproduction.

In these experiments, which were arranged to study the influence of reaction on the nitrogen-assimilating bacteria, 21 strains of *R. leguminosarum* and two of Azotobacter were studied. In general, *R. leguminosarum* showed similar cultural characteristics—that is, bacteria from different legumes. The most noticeable difference was that of rate of growth certain strains developing much more rapidly than others. On the ordinary culture media *R. leguminosarum* does not show any very characteristic growth. The identity of the

organism was studied for each strain and in every case the organism used to inoculate plants grown under sterile conditions effected inoculation.

In all of the tests the organisms were inoculated into 50-cc. portions of mannitol solution in 200-cc. Erlenmeyer flasks, the reaction changed by the addition of sulphuric acid or sodium hydroxid, and the cultures incubated for four weeks at 28° C. At the end of the period of incubation the presence or absence of the bacteria was determined by plate counts, microscopical mounts, and by inoculation of mannitol-agar slants. Aside from the total acid or alkali, the hydrogen-ion content in these cultures was measured by the colorimetric method.

The results of these experiments show clearly that sulphuric acid in culture solutions is far more injurious to alfalfa bacteria than to lupine bacteria. In other words, the nodule bacteria from different plants behave differently toward acid. The legume bacteria may be divided into groups about as follows:

1. Critical P_H 4.9 .... Alfalfa and sweet clover.
2. Critical P_H 4.7 .... Garden pea, field pea, and vetch.
3. Critical P_H 4.2 .... Red clover and common beans.
4. Critical P_H 3.3 .... Soybeans and velvet beans.
5. Critical P_H 3.15 .... Lupines.

The alfalfa organism is the most sensitive of the legume bacteria to acidity, and, conversely, the lupine organism is the most resistant to acidity.

The toxicity of sodium hydroxid toward legume bacteria is not noticeable until the alkali is added in large amounts; approximately 10 times as much normal alkali as normal acid is required to produce a similar injury. The organisms from the nodules of different legumes failed to show any decided difference in respect to alkali. For instance, it appears that the alkali limit of growth is the same for *Rhizobium leguminosarum* from lupine or from alfalfa.

One striking fact noted in the data of these experiments is the extreme sensitiveness of Azotobacter to slight changes in reaction. As compared with the legume bacteria, this organism is far more sensitive. The acid limit of growth in mannitol solution for Azotobacter is about $N/1{,}333.3$ and the alkaline limit about $N/1{,}000$, or the critical P_H acid value 6.5 and the alkaline value 8.6.

In relation to hydrogen-ion concentration of medium the nodule bacteria from different legumes show a very decided difference. The evidence supports the conclusion that a correlation exists between the acid resistance of the bacteria and the acid resistance of the higher plant.

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SUSCEPTIBILITY AND RESISTANCE TO CITRUS-CANKER OF THE WILD RELATIVES, CITRUS FRUITS, AND HYBRIDS OF THE GENUS CITRUS [1]

[PRELIMINARY PAPER]

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COOPERATIVE INVESTIGATIONS BETWEEN THE OFFICE OF CROP PHYSIOLOGY AND BREEDING INVESTIGATIONS, BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE, AND THE DEPARTMENT OF PLANT PATHOLOGY, ALABAMA AGRICULTURAL EXPERIMENT STATION

INTRODUCTION

While many observations (see Table I) have been made on the susceptibility and resistance to Citrus-canker (caused by *Pseudomonas citri* Hasse) of the more common commercial varieties and species of Citrus, no systematic comparison has, as yet, been attempted to determine the susceptibility and resistance to canker of the wild relatives, the more obscure species and varieties, and the hybrids of Citrus as a whole. Owing to the strict quarantine measures against Citrus-canker and the inability to get together a representative collection of plants because of the time and funds necessary, a comparison of this kind has been impossible. Fortunately, through the efforts of Mr. W. T. Swingle, we now have represented in this country one of the most complete collections of Citrus plants and their relatives to be found in the world.

Through the cooperation of Mr. Swingle, the writer has been able to obtain plants from this collection, representing a large part of the family Rutaceae, to which the genus Citrus belongs. Plants have been placed for this study in the greenhouses at Auburn, Ala., and in the isolation field in the vicinity of Loxley, Ala.

[1] Published with the approval of the Director of the Alabama Agricultural Experiment Station.

[2] The writer gratefully acknowledges his indebtedness to the following personnel of the Bureau of Plant Industry: Dr. K. F. Kellerman, Associate Chief, and Mr. W. T. Swingle, Physiologist in Charge, Crop Physiology and Breeding Investigations, for their cooperation and many helpful suggestions; Mr. D. C. Neal, Pathologist, for assistance rendered during the progress of the work; Mr. T. Ralph Robinson, Physiologist, Office of Crop Physiology, for his aid in the preparation of the manuscript; and Miss Wead, of the same office, for the preparation of the table and bibliography.

The results obtained from two sets of inoculation experiments in the greenhouse for the past five months have been of such a nature that their publication at this time is deemed advisable. It must be kept in mind that the results here reported are only of a relative nature, and in no case must they be considered absolute. This is especially true of those plants which are reported resistant. Of course, in the further search for varieties which are resistant to canker, the plants which showed marked susceptibility in the greenhouse experiments will be discarded.

EXPERIMENTAL METHODS

ORGANISM USED.—The organism (*Pseudomonas citri* Hasse) used in the inoculation experiments was isolated by Mr. D. C. Neal, by the ordinary dilution methods directly from soil taken under an infected grapefruit (*Citrus grandis*) near Fairhope, Ala., on July 20, 1917. Preliminary inoculations with this strain on grapefruit and Satsuma orange (*Citrus nobilis* var. *unshiu*) in the greenhouse proved that it was exceedingly virulent.

TYPE OF PLANTS USED.—Practically all of the plants used in the inoculation experiments were grown either from seeds or cuttings in the greenhouses at Washington, D. C. A few plants were included which had been budded on *Poncirus trifoliata* Raf., obtained from nurseries in Alabama. The size of the plants varied from 6 inches to 4 feet in height, and from those having a single stem to the large plants which had been cut back, with numerous shoots. The first lot of plants was received from Washington, D. C., on July 21, 1917, while the second shipment arrived on October 1, 1917. The plants were either cut back or were in a dormant condition when received; but by the time the first inoculation experiment was started, the majority were in excellent shape for infection. In discussing the results of the experiments the Crop Physiology and Breeding Investigations' (CPB) numbers used by Mr. Swingle are given so that the origin of the plants can be traced at any time by consulting his records.

METHOD OF INOCULATION.—As the plants varied in height, three sizes of inoculation cases were used. Care was taken in mixing the plants, so that all types were represented in each case. Just before setting the plants in the cases six punctures were made with a sterile needle on a mature leaf and an old leaf of each plant. The maturity of a leaf was judged by the size, condition, and to some extent by the number, size and pore length of the stomata. In a preliminary study it was possible to distinguish the different ages of the leaves by the methods used by Pool and McKay (*16*)[1] in their study of the species of Cercospora on beet. The results of the inoculation experiments were such that, unless stated in the discussion, no mention will be made of the infections occurring at the punctures.

[1] Reference is made by number (italic) to "Literature cited," p. 356–357.

At the time of the first inoculation, August 27, 1917, the plants in the two large cases were thoroughly sprayed with two 48-hour-old (100 cc.) cultures of *P. citri* in beef bouillon. A similar (100 cc.) culture was used for the smaller case. On September 12 the plants were again sprayed with half the amount of solution used above. The plants were removed on October 13 to a large screened case. The same day a second lot of plants were set in the cases and inoculated. These plants were again sprayed with 48-hour-old cultures of *P. citri* in beef bouillon on October 23 and November 1. The plants remained in the cases until January 13, when they were removed to the screened case where they are at the present time. Thus, the plants in Experiments I and II have been exposed to the Citrus-canker organism for a period of six months (February 27) and five months (February 12), respectively.

Conditions governing inoculations.—During the course of the experiments a relatively high temperature has been maintained. On one or two nights during the cold weather the temperature dropped to 40° F., but the night temperatures averaged 60° and higher. During the day a temperature of approximately 90° and higher has prevailed the greater part of the time. Occasionally it dropped to 70° and at other times was nearer 95°. However, with these variations, a temperature has been maintained which, with other factors eliminated, made possible a maximum amount of infection.

Practically a 100 per cent humidity was kept in the cases. About once a week the plants were given a thorough spraying with a strong water pressure, a measure that would assist in distributing the canker organism. After the plants were transferred to the screened cage, they were sprayed on an average of twice a week. This spraying was carried out early in the morning, so as to produce a condition somewhat similar to a heavy dew. So far as humidity was concerned, then, infection was possible practically all of the time. The facts that numerous spots developed and that leaves were defoliated by canker in the screened case during the winter proved that these conditions were ideal for infection.

Another point that might be mentioned is that the plants were exposed to rather strong sunlight, as only a very light shade was used on the glass of the house.

Early in the work it was found that, even though ideal conditions of temperature and humidity were supplied for infection, few or no canker spots developed if the plant was not in good growing condition. The largest number of spots naturally occurred on mature leaves which were still tender and of a light-green color. Few spots appeared on the young leaves, while spots developed on the old foliage of the more susceptible plants only. Thus, it is a hard matter to fix any definite scale of susceptibility or resistance for a comparison of the different plants, especially when so many types are represented. However, by comparing

the character of growth of the plants and the number and type of spots produced, one can make a comparison in a relative way.

It must be borne in mind, then, that all the inoculations were carried out in the greenhouse under conditions of high humidity and temperature. The condition of the plant also played an important rôle in its susceptibility and resistance to Citrus-canker. By a close study of the plants and by the number and type of the spots it is possible to determine in a relative way and make comparisons of the susceptibility and resistance of the plants used in these experiments. Unless otherwise stated, plants have been in good growing condition suitable in each case for developing infection.

SUSCEPTIBILITY OF WILD RELATIVES OF THE GENUS CITRUS

RUTACEOUS PLANTS NOT CLOSELY RELATED TO THE GENUS CITRUS

Xanthoxylum Bungei[1] Planch. (CPB 11180, seedling), II.[2]

A spreading, deciduous shrub or small tree native of North or Central China.

The plant (12 inches in height) was in fair shape during the early part of the experiment, as the new growth was young and in fine shape for infection. While blister-like swellings developed soon after inoculation in the case, no canker spots were produced. Isolation cultures made from these swellings yielded negative results.

Casimiroa edulis Lav. and Lex. White sapote (CPB 7923, seedling), II.

A large tree native of Mexico.

No spots of any description have appeared on the foliage, although it has been completely surrounded by badly infected plants.

Glycosmis pentaphylla DC. (CPB 2905, seedling), II.

A small shrub common to the Orient.

No canker spots have appeared on any of the leaves.

Claucena Lansium Skeels. Wampi (CPB 7936, seedling), II.

A low-spreading tree native to South China.

No spots of any description have developed on the leaves.

Chalcas exotica Millsp. (*Murreaa exotica* L.). Orange jessamine (CPB 7975A, seedlings), I. II.

A small tree commonly grown in greenhouses for ornamental purposes.

No canker spots have appeared on either plant.

RUTACEOUS PLANTS BELONGING TO TRIBE CITREAE

Subtribe Aeglinae (Hardshell Fruits)

Aegle Marmelos Correa. Bael fruit (CPB 7983, seedlings), I, II.

A tree native to North India, but widely cultivated in India and surrounding countries.

No canker has developed on the foliage.

Aeglopsis Chevalieri Swingle (CPB 7633 and 7772, seedling and cuttings), II and I, II.

A large shrub growing near the coast in tropical western Africa.

No spots of any description have appeared on the leaves.

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[1] The nomenclature used by Mr. W. T. Swingle and others in Bailey's Standard Cyclopedia of Horticulture is followed in the article. It is suggested that this work be consulted frequently in this connection so that the nomenclature and synonymy of the plants used in the experiments will be clearly understood.

[2] Roman numeral refers to the number of the inoculation experiment.

Chaetospermum glutinosa (Merrill) Swingle (*Aegle glutinosa*, Merrill). Tabog (CPB 7799, seedlings), I, II.

A small spiny tree, native to the Island of Luzon, P. I., of promise as a useful stock for Citrus.

While several oily swellings have appeared on the young leaves, no definite canker spots have occurred. Isolations made from the suspicious spots yielded negative results.

SUBTRIBE FERONINAE (HARDSHELL FRUITS)

Feronia Limonia (Corr.) Swingle (*F. elephantum* Corr.). Wood-apple (CPB 2763, seedlings), I, II.

A small spiny tree, native to India, Ceylon, and Indo-China.

The leaves have remained free from canker spots.

Feroniella lucida Swingle. Kavista Batu (CPB 7882, seedlings), I, II.

A small spiny tree, native to Java, somewhat resembling *Feronia Limonia.*

No spots have appeared on the leaves.

SUBTRIBE LAVANGINAE

Hesperethusa crenulata Roem. Naibel. (CPB 2759, seedling), II.

A slender tree native to the hills of India, Burma, and Indo-China.

No canker spots have developed on any of the leaves, although small spots caused by a fungus have been numerous.

Triphasia trifolia P. Wilson. Lime berry (CPB 2689A and 7780, seedlings), I, II, and II.

A small tree widely cultivated in tropical and subtropical regions as an ornamental.

These plants (10 to 18 inches) did not thrive well in the inoculation cases, and the lower leaves turned yellow and fell rapidly. However, new growth was produced quickly, so that young leaves were present at all times. No spots of any nature have developed on the leaves.

Severinia buxifolia Ten. (CPB 2760, cuttings), I, II.

A dwarf tree native to South China, introduced into Europe and recently into America.

The plants (8 to 12 inches), like *Triphasia trifolia*, did not thrive well in the cases, and defoliation was severe. However, new growth was present, and in good condition for infection. No spots of any kind have appeared on the foliage.

SUBTRIBE CITRINAE

NONEDIBLE FRUITS HAVING SESSILE PULP VESICLES WITH BROAD BASES[1]

Citropsis Schweinfurthii Swingle and M. Kellerman. African cherry orange (CPB 11260, seedling), II.

A small spiny tree native to Central Africa.

No spots of any kind have appeared on the leaves.

Atalantia citrioides Pierre. (CPB 7534, cuttings), I, II (2 plants).

A small spiny tree native to Indo-China.

These plants (6 to 10 inches) did not thrive in the greenhouse, the leaves defoliated rapidly, and in January all but one plant was discarded. However, new growth took place during the experiments, so that infection was possible. No canker spots appeared.

[1] In a recent conference with Mr. Swingle he stated that the subtribe Citrinae might well be split up into two groups to include plants having (*a*) nonedible fruits having sessile pulp vesicles with broad bases, and (*b*) edible fruits with stalked pulp vesicles.

EDIBLE FRUITS WITH STALKED PULP VESICLES

Poncirus trifoliata (L.) Raf. (*Citrus trifoliata* L.). Trifoliate orange (seedling, Alabama), II.

The trifoliate orange, which is a small spiny deciduous tree native to North China, is used to the exclusion of all other plants as a stock for *Citrus* spp. in Alabama. Hedges and trees are also widely scattered through the State. It has been fairly well established that Citrus-canker was imported from Japan into Alabama, directly and indirectly, on trifoliate seedlings. In Alabama it ranks next to grapefruit in susceptibility to canker in the field. With the gradual elimination of grapefruit growing from Alabama, the trifoliate orange becomes of major importance in the eradication of canker in that State.

Unfortunately the plant included in the experiment remained dormant, and no new growth developed. However, a few infections have occurred on the old leaves. Owing to the lack of good infections for comparison, the type of spots produced will be omitted.

Eremocitrus glauca (Lindl.) Swingle. (*Triphasia glauca* Lindl.; *Atalantia glauca* Benth.) Australian desert kumquat (CPB 7239, seedling), I, II.

A small hardy drougth-resistant tree with very small leaves and slender spiny twigs, found in Australia. It is one of the most interesting plants in Mr. Swingle's collection.

Typical Citrus-canker spots appeared on the leaves, twigs, and thorns six weeks after inoculation in Experiment I and in three months in Experiment II. Infection apparently takes place only on the upper surface of the leaves. The spots are minute, 0.3–0.5 mm. in diameter, circular, light brown at first, becoming darker with age, raised, compact, with little or no cork. The spots do not push through the leaf. Only an indistinct oily outline is present with a rather wide yellow zone. The spots increase very slowly with age, but one spot is sufficient to cause defoliation. The spots on the thorns and twigs are identical with those on the leaves.

It is extremely difficult to judge its relative susceptibility, owing to the peculiar nature of the plant. However, Citrus-canker attacks the leaves, thorns, and twigs, and as one spot is sufficient to cause defoliation, it is apparently quite susceptible.

Fortunella margarita (Lour.) Swingle. (*Citrus margarita* Lour.). Oval kumquat (CPB 7597, seedling), II.

The oval kumquat is quite widely grown in the Gulf coast section of Alabama. In the field kumquats have been reported as susceptible in only one or two cases, so that they are considered to be resistant by most growers.

As the plant (33 inches) was cut back for shipment, the young shoots were in excellent shape for infection at the time of inoculation. Citrus-canker spots appeared two months after inoculation and increased quite rapidly in numbers, so that practically all the mature leaves had one or more spots.

These results are quite interesting, in view of the resistance of kumquats shown in the field. The fact that maximum infection was obtained under the conditions governing the inoculations is very well shown here.

The spots are not numerous, averaging slightly above 1 mm. in diameter, chocolate-brown in color, slightly raised, compact, and occasionally corky. Spots rarely penetrate the upper surface, sometimes showing as a small, flat, oily, light-brown blister. The oily outline is very distinct, and no yellow zone is present. Citrus-canker does not cause defoliation, nor any apparent injury to the leaves. Only leaf infections have developed. Judging from the size and character of the spots, the plant is fairly resistant to canker, and it is not severe enough to cause any injury to the trees.

Fortunella crassifolia Swingle. Meiwa kumquat (CPB 11047, seedlings), I, II.

A little-known kumquat recently introduced into the United States by Japanese nurserymen.

In contrast to the fine condition of the oval kumquat, the plants (20 inches) are seedlings with a single shoot and have remained in a more or less dormant condition throughout the experiments. Consequently no canker has developed on these plants, even at the punctures, showing that the condition of the plants is of extreme importance in inoculation work of this character, and that unless all of the plants are in vigorous growing condition, no comparisons can be made. Inoculations with this kumquat will be repeated with more vigorous plants, and no doubt they will be found susceptible to some extent.

Fortunella Hindsii (Oliver) Swingle (*Sclerostylis Hindsii* Champ.; *Atalantia Hindsii* Oliver). Hongkong wild kumquat (CPB 11046C, seedlings), I, II.

This kumquat, which differs in some respects from the others, grows wild on the dry hills about Hongkong. Unfortunately no clear and concise test was made of this species, because the plants (12 inches) have remained in a more or less dormant condition throughout the experiments. Citrus-canker spots appeared early on the plant in the first experiment at four punctures on an old, tough leaf. Judging from the size and character of these spots, it will prove more susceptible than the oval kumquat. Inoculations will be repeated on more vigorous plants.

Microcitrus australasica (Muell.) Swingle (*Citrus australasica* Muell.). Finger lime (CPB 7600 and 7600B, cuttings and seedling), I, II, and II.

A small tree, native to the mountains of New South Wales and Queensland, Australia.

No spots of any kind have developed on the leaves.

Microcitrus australasica var. **sanguinea** Swingle (CPB 7775B, cutting), II.

A blood red variety of *M. australasica*.

No spots have appeared on the leaves.

Microcitrus Garrowayi (Bail.) Swingle (*Citrus Garrowayi* Bail.). Garroway's finger lime (CPB 11008, cuttings), I, II.

A plant similar to *M. australasica* and native to the same region.

A few oily swellings have developed which yielded negative results on making isolations.

Microcitrus australis (Planch.) Swingle (*Citrus australis* Planch.). Dooja (CPB 7307 and 7427, cutting and seedling), I, II.

An Australian lime, much more vigorous than the others, growing in the subtropical coastal forests of New South Wales and Queensland. The first plant (8 inches) developed very slowly and did not produce much new foliage. The second plant (32 inches), however, developed a large amount of new growth and has been in splendid condition for infection. Numerous Citrus-canker spots appeared on the leaves, twigs, and thorns of this plant one month after inoculation. Since that time canker has caused rapid defoliation of the plant, so that 50 per cent of the leaves have fallen. The plant is very susceptible. No doubt the other species of Microcitrus will prove susceptible to some extent when the right conditions are met with.

The spots (Pl. 53, G.) produced are numerous, small, occasionally 1 mm. in diameter, of a chocolate-brown color, raised, corky, and pushing through to the upper surface. Little or no oily outline is visible, while the yellow zone is wide and very distinct. Defoliation was rapid, and twig and thorn infection severe. The plant is quite susceptible, ranking slightly below *Poncirus trifoliata*.

From the results, up to March 1, 1918, of the inoculations in the greenhouse on the wild relatives of citrus, it appears that Citrus-canker is apparently limited to those plants having edible fruits with stalked

vesicles of the subtribe Citrinae. The susceptibility of the plants closely related to the genus Citrus is in the following order: *Poncirus trifoliata*, *Microcitrus australis*, *Eremocitrus glauca*, *Fortunella Hindsii*, and *Fortunella margarita*.

No doubt some of the other species and varieties of the wild relatives will prove susceptible when other tests are made with vigorous growing plants.

SUSCEPTIBILITY OF CITRUS FRUITS

Citrus Hystrix DC. (CPB 7872, seedlings), I, II.

A little-known group of plants found in the Philippine Islands, where they are sometimes used as a stock for Citrus spp. A characteristic plant with very large petioles.

Neither plant (20 inches) was in very good condition for infection; consequently Citrus-canker did not develop rapidly. However, spots appeared on the leaves and twigs, and some defoliation has resulted. Plants are not quite as susceptible as grapefruit.

The spots (Pl. 52, C) resemble those described on grapefruit in their general characters.

Citrus Hystrix Wester. "Cabayao" (CPB 7831, seedling), II.

A plant similar to the above. Owing to better condition of the plant (20 inches), citrus-canker has been much more severe. Apparently it is as susceptible as grapefruit. Infections are found on the leaves and twigs and some defoliation has resulted. The spots are identical with those on *C. Hystrix* 7872 (Pl. 52, C.).

Citrus Medica L. Citron of commerce (CPB 7768, cuttings), I, II.

Both plants (20 inches) pushed out an abundance of new growth while in the cases. Citrus-canker spots appeared on the leaves two weeks after the first inoculation. At the present time the spots are fairly well distributed over the leaves, and new infections are developing weekly. Canker is confined to the leaves and has not caused any defoliation.

The spots (Pl. 50, A) are fairly well distributed over the leaf, small, occasionally measuring over a millimeter in diameter, with no apparent increase in size. They are light brown at first, becoming darker with age. The spots are raised and somewhat corky, breaking through the upper surface and appearing flat and compact. The oily outline is distinct and is present only around unbroken spots, while the yellow zone is quite wide.

Several other species of *C. Medica* gave positive results, the order of susceptibility being as follows: "Etrog" citron 11178, "Sidro" citron 7816, citron of commerce 7768, "Nana" citron 11281, citron 7836, "Odorata" citron 11294.

The same type of spot (Pl. 50, A) was found on all the plants, and in some cases where they were extremely numerous on a leaf caused defoliation. However, owing to the small size of the spots, no injury resulted when they were scattered over the leaf. The number of spots per leaf does not influence their size. This point is important in judging the susceptibility of the citrons.

Citrus sp. Small lemon (CPB 7833, seedling), II.

An introduction from the Philippines.

The new growth has been excellent and consequently in fine shape for infection. Citrus-canker appeared on the foliage early in the experiment and has spread very rapidly to all the leaves, causing considerable defoliation. Numerous spots are present on the upper surface of the leaves. Twig infections are rather severe. The plant is extremely susceptible.

The spots are very numerous, small to large, light brown, raised, compact, spreading, and corky. The spots break through to the upper surface and are raised, corky, and spreading. Oily outline is distinct, while the width of the yellow zone varies. Very much like spots (Pl. 50, B) on grapefruit in general character. Spots different from those on citron in a number of particulars, but especially in size and appearance on upper surface.

Citrus sp. Sweet lemon (CPB 1158, seedlings), I, II.

An introduction from Jaffa, Palestine.

Both plants (16 inches) have been in fine condition for infection. Citrus-canker appeared on the foliage shortly after the first inoculation and has spread rapidly. Infections occurred on both surfaces of the leaves, and on the twigs and the thorns. It has caused defoliation of the more badly infected leaves. The plants are extremely susceptible.

The spots (Pl. 53, H) are very numerous, small to medium size, light-brown to brown, raised, more or less compact, with some cork. The spots cause a dead depressed area on the upper surface, but do not break through. An oily outline is present, while the yellow zone is indistinct, except when the spots coalesce. The spots are like those on citron except in size.

Citrus sp. "Davao lemon" (CPB 7837, seedling), II.

An introduction from the Philippines.

The leaves of this plant (18 inches) have the texture of citron leaves, with the shape and petiole of lemon leaves. The new growth has been fine, and in excellent shape for infection. Citrus-canker appeared early in the experiment and has spread rather rapidly over the foliage, so that infection has been rather heavy. The plant is susceptible to a marked degree, although not so much as some of the other lemons. The character of the spots (Pl. 53, I) is like that on the citron except in size. This may be expected, owing to the citron-like texture of the leaf.

Citrus sp. Limon real 18 (CPB 7819, seedling), II.

The leaves of this plant (18 inches) have the texture of citron leaves, with the shape and petiole of lemon leaves. The new growth has been fine, and was in excellent shape for infection. Citrus-canker appeared early in the experiment, and has spread rather rapidly over the foliage, so that infection has been rather heavy. The plant is susceptible to a marked degree, although not so much as some of the other lemons.

The character of the spots (Pl. 50, D) are like those on the citron. This may be expected, owing to the citron-like texture of the leaf.

Citrus sp.(?). Ichang lemon (CPB 11291, seedling), II.

Introduction from Hankow, China.

Judging from the character of the leaves, this is not a true lemon, but possibly is a hybrid. The leaves are dark green, smooth, pointed at the apex, with a large winged petiole. Occasionally a small leaflet arises from the point of union between the leaf and petiole. More or less pummelo-like in character.

The plant (10 inches) has been in good shape for infection. Citrus-canker appeared early in the experiment and spread rather rapidly. This plant is about as susceptible as grapefruit.

The spots are not numerous, but large, light brown, slightly raised, spreading, with cork present, breaking through the upper surface, forming a flat, spreading, compact spot. The oily outline is quite distinct, and the yellow zone is conspicuous. The spots are typical of those found on grapefruit leaves (Pl. 50, B).

Citrus aurantifolia (Auct.) Swingle (*C. limetta* Auct., not Risso.). Sour lime (CPB 7338, seedling), II.

The plant (18 inches) has been in fairly good shape for infection. Canker spots developed shortly after inoculation and have spread rapidly. At the present time the majority of the mature leaves are infected. While no defoliation has taken place, the plant is susceptible to a large degree.

The spots (Pl. 51, C) are numerous, small to medium size, rather dark brown, slightly raised, flattened on top, with some cork present. They break through the upper surface, forming either a small slightly raised spot or a depressed dead area. The oily outline is quite distinct. No yellow zone is present. The type of spot is rather characteristic and can not be compared directly to any of the other types discussed.

Citrus grandis (L.) Osbeck (*C. decumana* L.). Grapefruit (CPB 11170, seedlings), I, II.

The plants (12 inches) have been in only fair condition, so that infection was not as heavy as might be expected of grapefruit. However, spots have developed on the leaves and twigs. Some defoliation has taken place. The plants are extremely susceptible.

The spots (Pl. 50, B) are few per leaf, large (5–6 mm. in diameter), brown, raised, spreading, corky, breaking through the leaf and forming the same type of spot. The oily outline is very distinct and the yellow zone wide. A few spots on a leaf are sufficient to cause defoliation. The size of the spot is influenced somewhat by the number to the leaf.

Citrus grandis (L.) Osbeck (*C. decumana* L.). Grapefruit (budded on *Poncirus trifoliata*, Alabama).

As the plant (13 inches) was in poor shape for infection during the course of the experiment, only a few spots developed on the old leaves. However, they are typical of those on the preceding plants except in size. The degree of susceptibility of a plant can be judged somewhat by the number of spots occurring on the old tough foliage, as in the case of this plant.

Citrus grandis (L.) Osbeck (*Citrus decumana* L.). Pummelo (CPB 7834, seedling), II.

The leaf characters of this plant are very similar to grapefruit. However, the shape of the leaf differs somewhat, and the petiole is more winged.

The plant (13 inches) has been in excellent shape for infection, owing to the rapid growth of the young foliage. Citrus-canker has developed on the leaves, also along the midrib on the upper surface, twigs, and thorns. The plant is extremely susceptible, more so than grapefruit. Defoliation of the upper leaves by Citrus-canker has been rapid.

The spots (Pl. 51, B) are typical of those found on grapefruit in all details.

Citrus grandis (L.) Osbeck. Hirado Buntan (?). Pummelo. (CPB 7993, seedling), II.

A plant very similar to the preceding form, except that the petiole is slightly more winged.

As the plant (12 inches) made a rapid growth in the cases, Citrus-canker developed very early in the experiment. It is extremely susceptible, and defoliation by canker has been rapid. Apparently it is about as susceptible as pummelo 7834.

The type of spots (Pl. 50, B) produced is similar in all respects to those on grapefruit.

The identity of this pummelo as Hirado Buntan is in doubt as the plant numbers became mixed in transferring them from the greenhouses in Washington. In Japan the Hirado Buntan was noted by Mr. Swingle in 1915 as being decidedly canker-resistant.

Citrus nobilis Lour. "Naranjita"? (CPB 7830, seedling), II.

The leaf characters of this plant resemble those of the Satsuma.

Although the plant (14 inches) has ben in fairly good condition for infection, only one small Citrus-canker spot has developed. It will be tested again, as it gives some promise of being resistant to canker.

Citrus nobilis var. **unshiu** Swingle. Satsuma (budded on *Poncirus trifoliata*, Alabama), II.

Although the plant (36 inches) has not been in good condition for infection, Citrus-canker has developed on a number of leaves. However, infection was not severe and caused no defoliation or apparent injury to the leaves.

The spots (Pl. 52, E) are few in number, small, brown, raised, compact, with no cork present, breaking through to form a blister-like spot. The oily outline is distinct, with only a faint yellow zone. Resembles infections on *Fortunella margarita*.

Citrus mitis Blanco. Calamondin orange (CPB 11265 and 44305, seedlings), I, II, and II.

A hardy tree, native to the Philippine Islands, and commonly grown in Hawaii. Some years ago distributed by nurserymen in this country under the name "tokumquat."

The first two plants tested (15 inches) remained in a poor condition throughout the experiment, so that little or no Citrus-canker developed. However, the third plant (20 inches) has been in good condition for infection. Canker has been rather severe on the plant and has caused some defoliation of the leaves. Spots are present on the upper surface of the leaves. Not as susceptible as grapefruit.

The spots (Pl. 52, A) are many, small to medium size, light brown, and raised, compact, corky, forming a depressed dead area on the upper surface. Oily outline is indistinct, while yellow zone is scarcely visible.

Citrus sp. "Naranja," native orange (CPB 7929, seedling), II.

A recent importation from Porto Rico. Leaf characters very much like a grapefruit.

The plant (15 inches) has been in fairly good shape for infection and Citrus-canker has been severe. About as susceptible as grapefruit.

The spots (Pl. 51, A) are fairly numerous, medium to large, light brown, raised spreading, corky, breaking through the upper surface to form a depressed dead area or a raised spreading spot. The oily outline is quite distinct, while the yellow zone is almost absent.

Citrus sp. Kansu orange (CPB 11242, seedling), II.

An interesting plant collected by Mr. Frank N. Meyer in North China and not yet described. Apparently very hardy.

The plant (20 inches) thrived very well in the greenhouse, and new growth has been abundant. While Citrus-canker has developed on most of the mature leaves, it is causing no injury to the foliage whatever, and it gives promise of showing considerable resistance to canker in this respect.

The spots (Pl. 52, B) are many, extremely small (0.3 mm.), and not increasing in size, dark brown, raised, compact, not corky, not breaking through to upper surface. In fact, Citrus-canker can not be detected from a glance at the upper surface of the leaves. The oily outline is absent, and there is not the faintest trace of a yellow zone.

Three species of Citrus known in the Philippines as "colo-colo" (CPB 7820), "talamisan" (Pl. 51, D) (CPB 7827), and "tegi-tegi" (CPB 7818) proved susceptible, resembling in type of infection, respectively, sweet lemon, grapefruit, and citron.

Citrus excelsa Wester (CPB 11280, seedling), II.

A citron-like plant recently introduced from the Orient.

Owing to the poor condition of the plant (10 inches), little Citrus-canker has developed. However, what few spots are present on the leaves resemble those on the citrons.

These results obtained show that all of the Citrus fruits are more or less susceptible to Citrus-canker. While it is true that the plants included in the inoculation experiments represent only a small portion of the species and varieties found in this group, apparently the only plants which show any marked resistance to Citrus-canker are those included under *Citrus nobilis* and the Kansu orange. *Citrus mitis*, which has been reported to be resistant to canker in the Philippines (*26*), can hardly be classed with the promising resistant forms under greenhouse conditions. However, tests with this species will be repeated both in the greenhouse and in the field.

The type of spots produced on the various plants are striking, and in many cases the plants can be classed from a botanical standpoint, and relationships traced by the character of the spot. The susceptibility of the plants can also be arranged by the number and type of the spots per leaf. The spots vary from 6 mm. in diameter on grapefruit (Pl. 50, B) to 0.3 mm. on the Kansu orange (Pl. 52, B). While the size of the spot is influenced to some extent by the number of spots on the leaf surface, apparently those plants on which the small spots are found are not as susceptible as those with the large spots, as in the case of grape fruit.

SUSCEPTIBILITY OF CITRUS HYBRIDS

Faustrime (*C. aurantifolia*, West Indian lime, × *Microcitrus australasica*) (CPB 49819 and 49823, cuttings), II.

These hybrids retain to a large extent the characteristics of species of Microcitrus.

The plants (12 to 18 inches) have not been in good shape for infection during the experiment. Not much new growth has developed, so that they have not had a good test.

Up to March 1, 1918, only two small spots (Pl. 53, F) have developed, one at a puncture and the second on a small leaf. *Microcitrus australasica* has remained resistant, so that it is interesting to note that the hybrid is very slightly susceptible.

Faustrimon (*C. Limonia*, lemon, × *Microcitrus australasica*). (CPB 49824 and 49843, cuttings), II.

The plants (6 to 8 inches) are smaller and resemble very closely *Microcitrus australasica*. The growth of the foliage has been very slow so that they have been in only fair shape for infection.

No spots of any kind have developed on the leaves.

Citrange, Colman (*Poncirus trifoliata* × *C. sinensis*). (CPB 7896, seedlings), I, II.

The plants (18 inches) have been in only fair shape for infection. However, Citrus-canker appeared shortly after the first inoculation (15 days) and spread quite rapidly over the mature leaves. During the colder weather, when it was impossible to maintain a high temperature, the plants defoliated quite badly, so that few infections have occurred during the last two months. This was true of all the citranges.

The spots (Pl. 53, B) vary in numbers per leaf from one to many. They are small to large (2–3 mm) (Pl. 53 B, E), light to dark brown, raised, somewhat spreading, and corky, breaking through to the upper surface to form a slightly raised spreading spot. The oily outline is distinct, while the yellow zone varies in width. This description characterizes the spots on all the citranges. All are typical of those found on the *Poncirus trifoliata*.

With the exception of the Willits citranges, the following citranges tested showed approximately the same degree of susceptibility as the Colman: Cunningham (CPB 7665); Morton (CPB 771A (Pl. 50, E) and 761AC); Rusk (CPB 7956, 11030, and 44980); Rustic (CPB 7934A); Sanford (CPB 7963); Savage (CPB 7961); citranges (CPB1416 43480, and 43491).

Citrange, Willits (CPB 7897, seedlings), I, II.

These plants (14 inches) have been in fine shape throughout the experiments and have retained their foliage. The leaves have held their dark-green color. While Citrus-canker is fairly well distributed over the foliage, it is the only citrange that gives promise of showing any resistance to canker.

The citranges as a whole, with the possible exception of Willits, are equally susceptible to Citrus-canker, and no doubt under field conditions they will probably show about the same susceptibility as *Poncirus trifoliata*. This is to be expected since it is known that both parents of the citranges are quite susceptible to canker.

The character of the spots are similar on all the plants and resemble those produced on *Poncirus trifoliata*. As all the leaves of the hybrids are trifoliate and of the same texture as those of the trifoliate orange, naturally the same type of spot would occur.

Citrumelo (*C. grandis*, Bowen grapefruit, × *Poncirus trifoliata*). (CPB 4493, 4554, 4564, seedlings), I, II, I, II, and I.

The plants (9 to 16 inches) varied a great deal in their condition for infection. On the whole, considerable new growth developed, and as a consequence Citrus-canker appeared early in the experiments and spread rapidly to the young and mature leaves. Spots are present on the leaves, twigs, and thorns, and have caused considerable defoliation on one or two plants. Apparently as susceptible as either parent, grapefruit or trifoliate orange.

The spots (Pl. 53, A) are numerous, medium to large, light to dark brown, raised, spreading, and corky, breaking through to the upper surface to form a slightly raised, corky and spreading spot. The oily outline is distinct, and the yellow zone varies in width. The spots resemble to some extent those found on both parents, but are not typical of either.

Citradia (*Poncirus trifoliata* × *C. Aurantium*, sour orange). (CPB 50850, seedlings), I, II.

The plants (10 inches) have been in only fair shape for infection, although new growth developed once during the experiment. Citrus-canker appeared shortly after inoculation at the punctures but spread slowly to the healthy foliage. However, new infections were noted at each monthly reading. Apparently the plants are quite susceptible but not as much so as either parent. No doubt this hybrid will prove as susceptible to Citrus-canker as the parents when more vigorous plants are inoculated.

The spots are few, small to large, light to dark brown, slightly raised, compact, with little cork, breaking through the upper surface to form a compact spot. The oily outline is distinct, and the yellow zone varies in width. The spots are more or less typical of those found on *Poncirus trifoliata*.

Citrandarin (*C. nobilis*, King of Siam orange, × *Poncirus trifoliata*). (CPB 40210, 40303, 40315, seedlings), I, II.

The plants (7 to 15 inches) have been in fair shape during the experiments and some new growth has developed. Citrus-canker appeared at the punctures, but spread very slowly on the healthy leaves, so that only a few spots developed on each plant. This hybrid shows some resistance to canker and is about as resistant as the Satsuma orange.

The few spots (Pl. 53, D) present are rather typical of those found on *Poncirus trifoliata*. This is probably due to the leaf texture, which is about the same as that of the trifoliate orange.

Cicitrange (*Poncirus trifoliata* × Colman citrange, and *Poncirus trifoliata* × Sanford citrange). (CPB 48290, 48316A, seedlings), I, II and I, II.

Most of the plants (8–20 inches) have been in fine shape for infection. Citrus-canker appeared shortly after inoculation and spread rapidly to the new growth, where it was very severe, causing considerable defoliation. The spots are present on the leaves, twigs, and thorns. The plants are as susceptible as the trifoliate orange. It is interesting to note that the citranges used in the crosses are both very susceptible. The spots (Pl. 53, C) are typical of those found on the trifoliate orange.

Citrangequat (Willits citrange × *Fortunella margarita*). (CPB 48010, seedlings), I, II.

These interesting plants (12 inches) have made a very slow growth while in the cases, but new growth has been present practically all the time. Even though the plants were set under plants literally covered with Citrus-canker, they have remained resistant. This hybrid is the most promising of all the hybrids so far tested. Further inoculations both in the field and greenhouse will be carried out with a number of plants under different conditions to test out this resistance.

It might be pointed out in this connection that the Willits citrange, which shows more resistance than any of the other citranges, was used as one of the parents of this hybrid.

Citranguma (*C. nobilis* var. *unshiu*, Satsuma, × Morton citrange). (CPB 48055A, seedlings), I, II.

The plants (8 to 10 inches) have made a slow growth in the cases and have not been in good condition for infection. No Citrus-canker has developed on the plants, which are apparently resistant, although both parents are susceptible, especially the Morton citrange, which is highly so. Further tests will be made with this hybrid.

Limequat (*C. aurantifolia*, West Indian lime, × *Fortunella japonica*, Round kumquat). (CPB 48787A, 48787B, seedlings), I, II.

The plants (8 to 12 inches) have been in good shape for infection. This was especially true during the first two months, when Citrus-canker was rather severe on the leaves. Some defoliation resulted on one or two of the plants. The limequat is not as susceptible as the lime and not as resistant as the kumquat. Apparently where limes are used as a parent we can expect the hybrid to be susceptible, even though the other parent is fairly resistant. The susceptibility of the limequat should be contrasted with that of the citrangequat, where both parents are fairly resistant to canker. The spots (Pl. 53, J), while not as numerous per leaf area as those on the sour lime, are identical in character, except in size.

Limelo (*C. aurantifolia*, West Indian lime, × *C. grandis*, sour pummelo). (CPB 40502, 40526A, 40567B, seedlings), I, II, I, II.

The plants (8 to 19 inches) have been in fairly good shape for infection. Citrus-canker appeared very early in the experiment and spread rapidly. Spots are present on the leaves, petioles, twigs, and thorns, and some defoliation has resulted. The plants are apparently as susceptible, or more so, than either of the parents.

The spots (Pl. 52, D) are typical of those found on grapefruit.

Tangelo, Thornton (No. 2) (*C. nobilis* var. *deliciosa*, tangerine, × *C. grandis*, Florida grapefruit).[1] (CPB L715A, seedlings), I, II.

The plants (10 to 12 inches) have not been in the best of condition for infection, as growth was slow. Citrus-canker developed shortly after inoculation at the punctures, but did not spread rapidly to the young foliage, so that at the present time only a few of the leaves are infected. While the spots (Pl. 50, C) are typical of those on grapefruit, the hybrid gives some promise of being much more resistant to Citrus-canker than grapefruit.

Tangelo, Sampson. (CPB L789A, seedlings), I, II.

In many respects these plants (12 inches) were similar to the Thornton tangelo in their growth and behavior toward Citrus-canker.

Tangelo (CPB 1230, seedling), I.

The plants (14 inches) were in about the same condition for infection as the Thornton tangelo and appeared to be slightly more susceptible.

Tangelo (CPB 1257A, seedlings), I, II.

The plants were similar to tangelo 1230 in growth and susceptibility to canker. The spots, like those of the other tangelos, are typical of those described for grapefruit.

The results obtained with the hybrids are extremely interesting and instructive. When two susceptible plants are used in hybridizing the hybrid shows the same susceptibility as the parents. Good illustrations of this are the citranges and cicitranges, citrumelos, and limelos. However, when an extremely susceptible plant is crossed with a kumquat or a mandarin type of orange the hybrid retains to a large extent the resistance exhibited by the resistant parent, as illustrated by the behavior of the citrangequat, citranguma, citrandarin, and to a less extent by limequat and tangelos.

Unfortunately, no hybrids were included in the experiments where both parents show marked resistance.

In the further work of hybridizing for resistance to Citrus-canker the parents must be confined for the most part to those plants which show marked resistance to canker, especially in the genus Fortunella and *C. nobilis* with its many varieties.

DISCUSSION OF RESULTS

The factors necessary for the successful inoculation of the plants in these experiments, especially those which were somewhat resistant, are a high temperature, a relatively high humidity, and a vigorous and rapidly growing plant. Without the inclusion of the last factor only the more susceptible plants are infected. It is for this reason that the condition of the plants for infection has been given for each host discussed. When the plants were not in good shape for infection, few or no results were obtained. In such cases no relative comparisons of susceptibility or resistance could be made.

Under the conditions governing the inoculations, then, the maximum amount of infection possible was obtained. No doubt under ordinary

[1] This is not the true Thornton, but a sister variety, differing considerably though resulting from the same cross.

field conditions the more resistant plants would show almost absolute immunity, while the less susceptible plants would show more resistance to Citrus-canker. For example, a larger number of canker spots were produced on the oval kumquat in the greenhouse than has been reported on all the kumquats in the field since Citrus-canker was introduced into this country. Where the plants were in good condition for infection and remained resistant we may rest assured that they will be resistant under ordinary field conditions.

The fact that the plants were thoroughly mixed in the cases also helped to produce the greatest amount of infection. The writer found that the organisms isolated from susceptible plants grow very rapidly on potato plugs, while those from the more resistant plants develop slowly.

It is also very difficult to isolate the organism from the spots on the more resistant plants. No doubt, if all the resistant plants had been placed together in a case and inoculated, little or no canker would have developed and no reinfections would have been possible. Thus, by mixing the plants a higher percentage of hosts were infected and reinfected, owing to the spread of the more virulent organisms from the extremely susceptible plants.

The results may be influenced somewhat by the fact that small, rather young plants, mostly derived from seedlings, were used. Just how much the individual variations of the seedlings enters into the work will not be known until a number of inoculation experiments are carried out.

These plants are natives of various parts of the world and grow under diverse climatic and soil conditions. When brought together in an experiment of this nature, undoubtedly many of them are weakened, a condition that influences their behavior toward Citrus-canker.

It is indeed surprising to find from the inoculation experiments in the greenhouse that, so far Citrus-canker is apparently limited to those plants having edible fruits with stalked pulp vesicles, of the subtribe Citrinae, which includes only five genera. Of these, the genera Poncirus, Fortunella, and Microcitrus were formerly included in the genus Citrus, while Eremocitrus was first described as a Triphasia and then as Atalantia before being placed in a new genus by Mr. Swingle. It is interesting to note how closely susceptibility to Citrus-canker under greenhouse conditions ties up with the botanical classification of this group of plants as worked out by Mr. Swingle.

Jehle (*10*) (*11*) has reported *Xanthoxylum fagara* (L.) Sarg., *X. Clava hercules*, and *Chalcas exotica* Millsp. (*Murraea exotica*, L.) as being susceptible to Citrus-canker. His method of inoculation (needle pricks) and the fact that the plants were kept in a tightly screened cage (*8*) into which no direct sunlight could penetrate,[1] might make his results possible.

In the Philippines, Wester (*29*) has noted "what is apparently Citrus-canker" on *Chaetospermum glutinosa* (*Aegle glutinosa* Merrill). No

[1] Sides and top were double-screened on the outside with galvanized netting and on the inside with bronze screen of fine mesh, with a mote at the base.

isolations were made of this material, so that this observation has not yet been definitely substantiated.

These are the only Citrus relatives so far reported in the literature as being susceptable to Citrus-canker. It should be noted that the relatives mentioned in this connection are widely removed from Citrus, and if these are as susceptible as reported, we should surely anticipate results with the closely related plants as Citropsis and Atalantia, which belong to the same subtribe. Further inoculation in the greenhouse and field this season should go a long way in clearing up this situation.

Of the relatives *Poncirus trifoliata* is undoubtedly the most susceptible, followed by *Microcitrus australis*, while *Eremocitrus glauca*, *Fortunella Hindsii*, and *F. margarita* show some resistance to Citrus-canker. No doubt some of the plants which did not become infected will probably prove susceptible to some extent when good vigorous plants are used.

All of the species and varieties of true Citrus proved susceptible. The citrons, lemons, lime, grapefruits, pummelos, and similar plants are so susceptible that in the search for resistant forms they can be discarded, unless other pummelo strains possessing resistant qualities may be found. The plants belonging to the species *C. nobilis* and the Kansu orange are the only forms which exhibited any resistance to Citrus-canker. It is from this group and type of plants that resistant forms will be obtained in the future.

The results obtained with the hybrids have by far been the most interesting and instructive. Two of the hybrids, the citrangequat and citranguma, have thus far remained completely resistant, while the other hybrids having the mandarin type of orange for one parent have shown considerable resistance.

When susceptible plants like grapefruit and lime have been used in crossing, the resulting hybrid generally retains this susceptibility, while the resistant parents pass their resistance on to the hybrid. The most far-reaching results in the search for commercial resistant varieties will be obtained in the development and hybridizing of the forms which show some resistance to Citrus-canker.

The type and number of spots varies directly with the resistance exhibited by a plant. This offers a means of judging and comparing the relative susceptibility and resistance of the whole group. Apparently resistance is in part mechanical—for example, the texture of the leaf determines to a large extent the size and character of the spot. Leaf texture plays an important role in the resistance of the host plant to Citrus-canker and seems closely related to the rapidity with which the leaves mature. There is a considerable variation in the time required for the maturation of the leaves of the various Citrus plants. Thus, the leaves of the kumquat, which are rather thick and highly resistant, reaches maturity much sooner than the thin, extremely susceptible leaves of the grapefruit.

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TABLE I.—*Relative susceptibility of different varieties of Citrus fruits to Citrus-canker, arranged according to dates of articles from which the table is compiled* [a]

[xxx, very susceptible; xx, susceptible; x, some resistance; o, decidedly resistant; oo, immune]

Species.	Stevens (*18*)	Wolf (*32*)	Stirling (*23*)	Stevens (*19*)	Edgerton (*4*)	Beattie (*1*)	Massey (*14*)	Stevens (*22*)
Grapefruit	xxx	xxx	xxx(1)	xxx	xxx	xxx	xxx	xxx
Orange:								
Sweet	oo	x	xx(6)	xx	xx			
Navel			xx(5)	xx				x
Satsuma	x	xx	xx(7)	xx	xx	o		
Mandarin			xx(9)					
Tangerine			oo(8)	xx				
King			xx(10)					
Trifoliate	xx	xx	xxx(2)	xxx	xxx		xxx	
Lemon			xx(11)	xx				
Lime			xx (3, 4)	xx				
Kumquat		oo	oo	oo	o			

Species.	Berger (*2*)	Swingle (*24*)	Stevens (*20*)	Stevens (*21*)	Rorer (*17*)	Kellerman (*12*)	Wolf (*30*)	Doryland (*3*)
Grapefruit	xxx(1)		xxx [b]	xxx	xxx(1)	xxx	xxx	xx
Orange:								
Sweet	xx(5)		xx	x	xx(5)	xxx	xx	xx
Naval	xx(4)		x		xx(4)			
Satsuma	xx(6)		x		x(6)	xxx	x	
Mandarin	xx(8)		x		x(7)		x	
Tangerine	xx(7)		x				x	o
King	xx(9)		x		x(8)			o
Trifoliate	xx(2)		xxx	xxx	xxx(2)		xxx	
Lemon	xx(10)		xx		x(9)	xxx	x	
Lime	xx(3)		x		xx(3)	xxx	x	
Kumquat	oo	xx		oo			x	

Species.	Edgerton (*5*)	Wolf (*31*)	Mackie (*13*)	Jehle. (*6*)	Jehle. (*7–9*)	Newell (*15*)	Wester (*26–29*)	Fed. Hort. Bd. (*25*)
Grapefruit	xxx	xxx	xxx	xxx(1)	xxx(1)	xxx(1)	(c)	(d)
Orange:								
Sweet	xxx	x		x(5)	x(7)	x(7)		
Navel	xxx	x		xx(4)	x			
Satsuma	xx	o		x(6)	x			
Mandarin	xx	x		x(8)	x			
Tangerine	xx	x	xx	x(7)	x(8)	x(8)		
King		x		x(9)	x(9)	x(9)		
Trifoliate	xxx	xxx		xxx(2)	xx(4)	xx(4)		
Lemon		x	xxx	x(10)	xxx(2)	xx(2)		
Lime		x	o	xx(3)	xx(3)	xx(3)		
Kumquat	o	x		o	o(11)	o(11)		
Miscellaneous [e]								

[a] Numbers in parentheses within columns indicate the relative order of susceptibility.

[b] Stevens (*20*); Inoculations on sweet orange, trifoliate, rough lemon, and grapefruit show about same degree of susceptibility to infection where growth and moisture conditions are the same.

[c] Wester (*26–29*): Mr. P. J. Wester has reported observations on the occurrence of canker under natural conditions at Lamao Experiment Station, near Manila, P. I. The collection of Citrus plants there includes about 1,000 separate numbers and embraces practically all the species of citrus being grown commercially in the United States, as well as many native and Asiatic forms, not commonly grown in this country. In addition to the recognized varieties (and some natural hybrids) observations were made on a large number of hybrids of the tangelo type furnished by the United States Department of Agriculture in connection with Crop Physiology and Breeding Investigations. The notes as to canker susceptibility showed in some cases results varying with the season of the year when observations were made and doubtless chance infection would have an influence as well. These observations are too detailed to report in this brief summary, and later examinations would probably make some changes necessary. It is significant, however, to note that there is a wide range of susceptibility under conditions favorable to the unrestricted spread of canker, some of the mandarin types of oranges being practically immune, while some of the tangelos showed marked resistance. Grapefruit and oranges of American origin are generally quite susceptible, while certain of the pomelos of Asiatic origin are reported as distinctly resistant.

[d] Federal Horticultural Board (*25*): Canker found on specimen of grapefruit and other Citrus species from Java and on five specimens from Japan.

[e] Miscellaneous varieties: Sour orange and tangelo are somewhat susceptible according to Jehle (*6–9*) and Newell (*15*). Mandarin limes are very resistant though not immune according to Jehle (*7–10*) and Newell (*15*). *C. pseudolimonum, C. l. aromatica, C. longispina, C. L. davaoensis*, and *C. webberii* are especially subject; *C. n. papillaris, C. mitis* and *C. w. montana* are practically immune according to Doryland (*3*). *C. micrantha* is quite susceptible according to Mackie (*13*).

From the results of the experiments it is a reasonable assumption that the virulence of the organism can be increased or decreased by a choice of hosts, just as the growth of the organism can be influenced on artificial media by giving it a favorable or unfavorable media on which to develop. By this means a very virulent strain can be built up by keeping it on grapefruit, while a virulent strain can be broken down by using kumquat as a host. There may be in nature different strains of canker organisms, and no doubt these do occur, but apparently these can be decreased or increased in virulence by the use of a susceptible or resistant plant, so that it all becomes a question of host relation.

SUMMARY

(1) Plants representing the more important wild relatives, species, varieties, and hybrids of Citrus were obtained from the United States Department of Agriculture and inoculated with *Pseudomonas citri* in the greenhouse to test their comparative susceptibility and resistance to Citrus-canker.

(2) The conditions under which the experiments were carried out were such that a maximum amount of infection was possible. The factors influencing infection were a high temperature, a relatively high humidity, and a rapid and vigorous growing plant.

(3) From the results of the greenhouse inoculations with young plants, Citrus-canker is apparently limited to those plants having edible fruits with stalked pulp vesicles of the subtribe Citrinae, which includes the genera Poncirus, Fortunella, Eremocitrus, Citrus, and Microcitrus.

(4) Susceptibility and resistance to Citrus-canker follow closely the botanical classification of this group as worked out in recent years by Mr. W. T. Swingle.

(5) Of the so-called relatives, the plants belonging to the genera Fortunella, Eremocitrus, and Microcitrus show some resistance to Citrus-canker, while Poncirus is extremely susceptible.

(6) All the species and varieties of Citrus tested are susceptible to canker. *Citrus nobilis* with its many varieties and types, the Kansu orange, and possibly *C. mitis*, exhibit enough resistance to warrant trials under Citrus-canker conditions in the field.

(7) Of the hybrids the citrangequat and citranguma have remained free from Citrus-canker in these tests, while the citrandarins, limequats, and tangelos show some resistance. The citranges, with the possible exception of Willits, cicitranges, citrumelos, and limelos are all extremely susceptible and can be discarded in the further search for resistant plants.

(8) The number, size, and character of spots on the leaves are of great assistance in judging the relative susceptibility and resistance of the plants.

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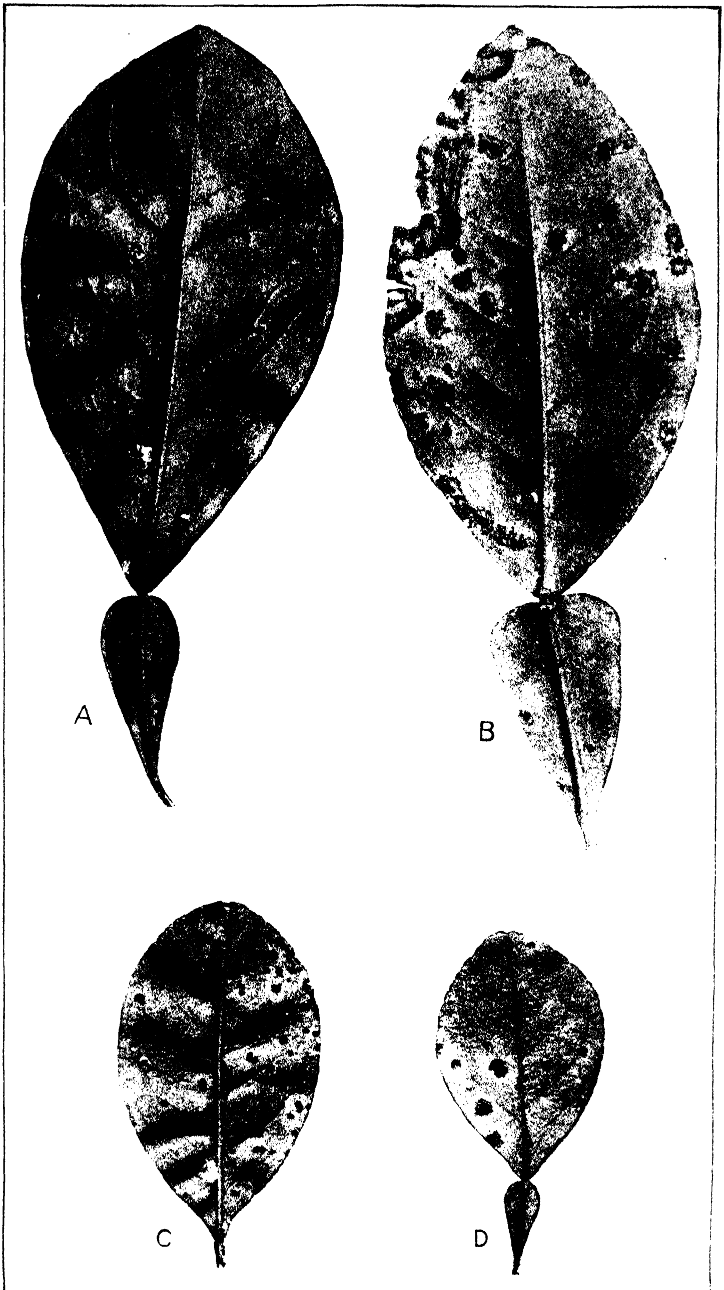
PLATE 50

Plates 50–53 illustrate the results of Citrus-canker inoculations in the greenhouse, showing the characteristic type and number of spots on the various plants. The spots are all representative of the particular host, approximately the same age and natural size.

A.—*Citrus Medica*, citron of commerce (CPB 7768);
B.—*Citrus grandis*, grapefruit (CPB 11170);
C.—Thornton tangelo (CPB L-715 A);
D.—*Citrus* sp., limon real 18 (CPB 7819).



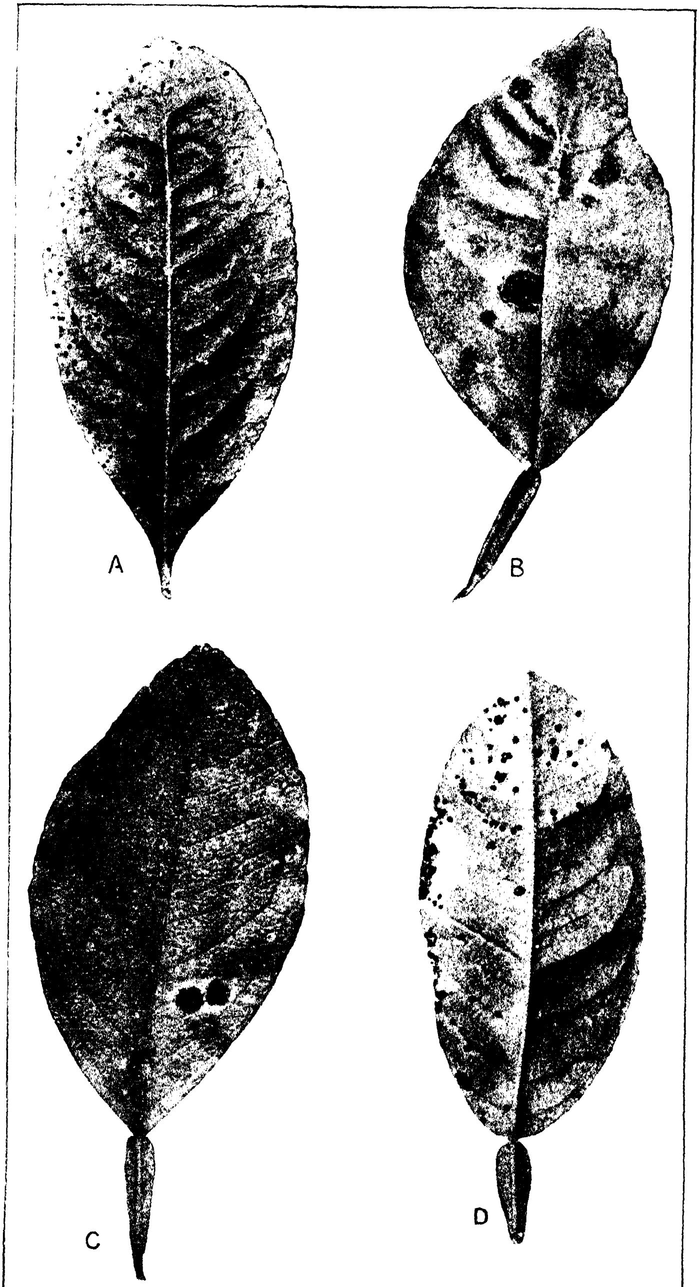

A
B
C
D

PLATE 51

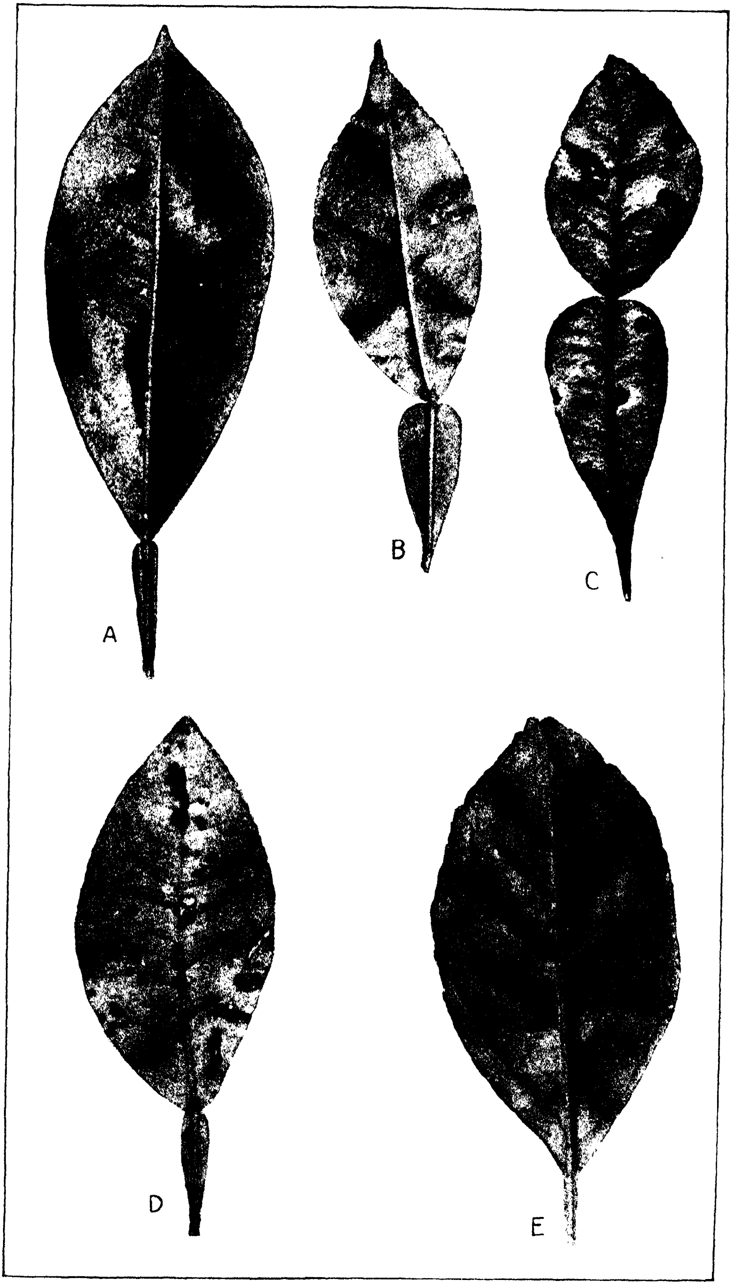
Results of Citrus-canker inoculations:

A.—*Citrus* sp., "naranja," native orange (CPB 7929);
B.—*Citrus grandis*, pummelo (CPB 7834);
C.—*Citrus aurantifolia*, sour lime (CPB 7338);
D.—*Citrus* sp., talamisan (CPB 7827).

PLATE 52

Results of Citrus-canker inoculations:

A.—*Citris mitis*, Calamondin orange (CPB 44305);
B.—*Citrus* sp., Kansu orange (CPB 11242);
C.—*Citrus Hystrix* (CPB 7872);
D.—Limelo (CPB 40567B);
E.—*Citrus nobilis* var. *unshiu*, Satsuma.


A
B
C
D
E

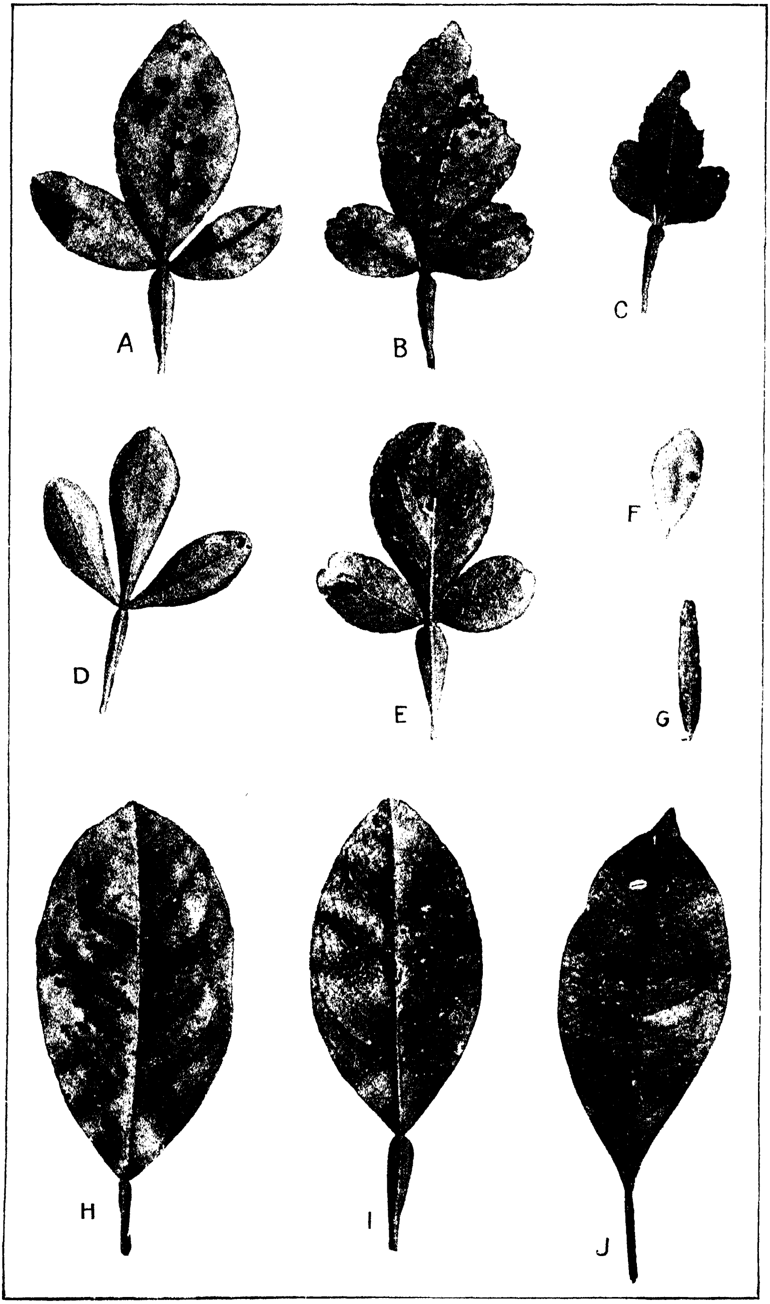

A
B
C
D
E
F
G
H
I
J

PLATE 53

Results of Citrus-canker inoculations:

A.—Citrumelo (CPB 4493);
B.—Colman citrange (CPB 7896);
C.—Cicitrange (CPB 48316A);
D.—Citrandarin (CPB 40210);
E.—Morton citrange (CPB 771A);
F.—Faustrime (CPB 49819);
G.—*Microcitrus australis* (CPB 7427);
H.—*Citrus* sp., sweet lemon (CPB 1158);
I.—*Citrus* sp., Davao lemon (CPB 7837);
J.—Limequat (CPB 48787B).

VARIATION AND CORRELATION IN WHEAT, WITH SPECIAL REFERENCE TO WEIGHT OF SEED PLANTED[1]

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INTRODUCTION

Extensive work has been done to determine the relative value for planting of seeds of various sizes and weights selected by the use of the fanning mill and by hand. Some work along this line has been done by weighing the individual seeds planted. The evidence from some of these experiments is inconclusive, and a study of them raises several questions regarding the seed used, the weather conditions, and the character of the soil for the different seasons, and the technic followed.

(1) Were the differences in weights or sizes of the individual seeds sown sufficiently great in any particular experiment so that a significant variation in yield could be expected?

(2) Were the desired stands of plants usually secured and were they such that the various grades of seed could give expression to their particular value?

(3) May not the rainfall and temperature conditions during any part or throughout the entire growing season have been such that differences in yield, which in all probability would have resulted under ordinary conditions, did not materialize?

(4) What has been the rôle of degree of fertility of the soils on which these trials have been conducted?

(5) Under the conditions which obtained for any particular year was the technic of the experiments such that the experimental error could be ascertained?

These factors, and in some cases others, are necessary considerations in arriving at conclusions from experiments regarding the relative value of various weights of seed for planting.

The data presented in this paper are the results of a 4-year preliminary study of size of individual seeds of wheat in their relation to the resultant plants, to aid in interpreting more accurately trials of similar nature which are now in progress under field conditions.

A careful study of the reactions to environment over a period of years of plants grown from accurately weighed seeds of various sizes ought to give fundamental information of value in this connection.

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In order to obtain the desired data in a form comparable for the 4-year period and easily presented, the biometrical method was used (*Davenport, 1907*).[1]

The subject matter is arranged for presentation in two main divisions. In the first division variability both of the seed used and of the resulting plants is studied. The means, standard deviations, and coefficients of variability are used to this end. In the second division (1) degree of relation between weight of seed and characters of the resultant plants, and (2) degree of interrelation between characters of the resultant plants are shown by correlation coefficients.

REVIEW OF LITERATURE

Love and Leighty (*1914*), working with a pure line of oats, found that biometrical constants—that is, means, standard deviations, coefficients of variability, and correlation coefficients—vary more or less with environmental conditions, such as degree of crowding of the plants and differences in the weather conditions. With conditions not so favorable for plant development, less variability was found in number of culms, total and average number of spikelets, and average number of kernels per spikelet. In average weight of kernels, greater variability was found under unfavorable conditions. When development was arrested by environmental conditions, yield was lowered, not by reduction in average weight per kernels or number of spikelets produced, but by a reduction of the number of kernels per plant, per culm, and per spikelet. Correlations were broadly classified as (a) fluctuating, which vary considerably with environmental conditions, and (b) stable, which vary less from year to year. Between yield of kernels per plant and their average weight no correlation was found in one trial and in two others the coefficients were low—but five and seven times their probable error, respectively. The interpretation is that, for the years when correlation between these two characters occurs, selecting the largest seeds would be securing them from the heaviest yielding plants. Average height of plants, as correlated with average weight per kernel, gave coefficients of 0.219 ± 0.029, -0.023 ± 0.034, and 0.217 ± 0.032, respectively, for three years. For one year there is no correlation. For the two other years the coefficient of correlation is seven times its probable error, which is significant. For the two years the taller plants had a tendency to produce the larger kernels.

Leighty (*1914*) found practically the same correlation coefficients when determinations were made on single culms as when whole plants were used. There was a uniform tendency for the coefficients to be greater when single culms were used. In studying oats grown in hills as compared with that growth in drills rather large differences occurred

[1] Bibliographic citations in parentheses refer to "Literature cited," p. 391-392.

in the correlation coefficients of the same variety for plants grown in the two ways. In any variety, when considerable differences occurred in the coefficients as obtained by the two methods, the lower was secured by using the plants grown in hills. From this it is concluded that differences due to spacing may amount to more than varietal differences.

Variability of yield, number of kernels, number of spikelets, and breaking strength of straw decreased with crowding, while for height the least variability occurred in hills.

Hutchinson (*1913*), in a statistical study of oat plants grown from individually weighed seeds planted at definite distances apart, found medium high positive correlations between weight of seed planted and each of the following characters: Yield of kernels, total weight of plant, number of kernels harvested, height before second leaf, height at 4, 6, and 10 weeks, at heading, and at harvest. No correlation was found between weight of seed planted and average weight of seed harvested. In two trials medium to high positive correlation was found between yield of grain per plant and average weight of kernels harvested, while in another trial no correlation was found between these two characters.

Atkinson (*1912*), using culms as the basis of determinations in a statistical study of eight spring-wheat populations grown under field conditions, obtained correlations as follows: Between yield and average weight of kernel a range from 0.508 ± 0.022 to 0.837 ± 0.009, between average weight of kernel and length of culm a range of from 0.098 ± 0.030 to 0.523 ± 0.022, and between yield and length of culms a range of from 0.217 ± 0.032 to 0.863 ± 0.008.

Meyers (*1912*), investigating the effect of soil fertility on variations and correlations in wheat, found that variability was decreased by increase in fertility, and that all correlations were greatest on the poorer soil.

Roberts (*1912*), working with three pure lines of wheat, found that variability was reduced in favorable growing seasons and concludes that seasonal and soil factors are probably sufficient to overcome hereditary distinctions of yield in good seasons.

Waldron (*1910*), in a statistical study of oats grown under field conditions, found in correlating average weight of kernels with number of kernels per plant a negative correlation of -0.595 ± 0.013. Between average weight of seed and length of head and average weight of seed and length of culm correlation coefficients of -0.511 ± 0.005 and -0.404 ± 0.017, respectively, were found. These correlations indicate that the large kernels are borne by the short plants having short heads and producing a small number of kernels per head.

Love (*1912*), on the other hand, shows positive correlation between height of plant and yield, between height and average weight of kernels, and between yield and average weight of kernels.

Montgomery (*1912*) sowed wheat and oats at different thicknesses and found that, when large and small seeds were planted together and the plants from them grew under competitive conditions, the highest mortality was among the plants from the small seeds. This indicated that the larger seeds produced the stronger plants. It was also found that, under field methods of seeding, there was a reduction of 40 per cent in the stand from planting time to harvest, even when large seeds only were used. The conclusion regarding size of seed is that, since under usual methods of thick seeding a high mortality occurs, it does not seem that fanning-mill selection can increase the efficiency of seed. In comparing two varieties of winter wheat having three grades of seed, lightest light, heaviest heavy, and the seed as it came from the thresher, no difference was found in quantity or quality of grain produced. A similar trial with one variety of oats gave like results.

Kiesselbach and Helm (*1917*) planted hand-selected, large and small seeds alone and in competition with each other. The yield of grain was 11 per cent lower when the small seeds were planted alone and 24 per cent lower when planted in competition with the large seeds. In a 2-year trial of hand-selected large and small seeds of two winter-wheat varieties compared with unselected seed the yield from the large seed was 2.3 per cent greater than that from the unselected seed and 5.4 per cent greater than from the small seed. In a similar trial with two varieties of spring wheat the yield of grain from the large seed was 11.8 per cent greater than that from the unselected seed and 19.5 per cent greater than the yield from the small seed. In these two trials the seed was sown in equal numbers at a normal rate for the large seeds. In a 1-year trial plants from small seeds spaced 6 by 10 inches produced 72 per cent as large a yield of grain as plants from large seeds similarly spaced. As an average for a 4-year trial of large, small, and unselected seeds of Turkey winter wheat and similar trials of Kherson oats covering a 5-year period, and Scotch Fife spring wheat covering a 2-year period, the small seed yielded one-third of 1 per cent less than the large seed when equal weights of seed were sown and 8 per cent less when equal numbers of seeds were sown. In a 12-year trial of the heaviest one-fourth and lightest one-fourth of continuous fanning-mill selected seed sown at the rate of 5 pecks per acre as compared for yield of grain with unselected seed of two varieties of winter wheat considerable variation occurred, but the average results show practically no difference. A similar trial of the same duration with Kherson oats gave somewhat higher yield for the lightest one-fourth as compared with the heaviest one-fourth 6 years out of 12, but the average is slightly in favor of the heaviest one-fourth. In a similar trial with American Banner oats, covering a period of 8 years, the lightest one-fourth yielded higher than the heaviest one-fourth in 6 out of 8 years and averaged 3.67 bushels more for the period of the trial.

Williams and Welton (*1913*) compared the yields for a period of 5 years from large and small seed oats separated by a fanning mill and sown at both a uniform rate in pounds per acre and at a varied rate, the aim of which was to secure the same number of plants per acre. The large seed exceeded the small in yield at both rates of seeding by approximately 4 bushels per acre. The experiment was continued by using large and small seed compared with seed as it came from the threshing machine. The 4-year results show no advantage in favor of the heavy over the ungraded seed at either rate of seeding. At the uniform rate of seeding the small seed was as efficient as either of the two other grades, but at the varied rate it produced 2 bushels less per acre. A comparison was also made of hand-selected primary and secondary kernels of oats definitely spaced. In 3 out of the 5 years the primary seeds proved more efficient.

In a more recent experiment Williams (*1916*) made a comparison in field trials for 8 years of large, small, and unscreened seed of winter wheat with no advantage of large over either of the two other grades. A 6-year trial of hand-selected, large and small seeds from pure lines of wheat showed an advantage in yield of 48 per cent in favor of the former.

Georgeson, Burtis, and Otis (*1897*) in an 8-year trial of three grades of seed oats, heavy, light, and unscreened, found the heavy seed more efficient than unscreened seed by 1 bushel and more efficient than the light seed by 3 bushels per acre.

In an earlier bulletin Georgeson, Burtis, and Otis (*1896*) report the results of a 6-year trial of heavy, light, and unscreened wheat. The heavy and unscreened seed gave practically the same yields, which were superior to the yields from the light seed by 1⅓ and 1½ bushels, respectively.

Zavitz (*1915*) reports the results of six trials with hand-selected, large and small seeds of four varieties of oats grown at seven distances apart. In 90 per cent of the trials the large seeds proved superior. In another trial, covering a period of from 3 to 9 years, hand-selected, large, plump seed yielded in oats 15.4 bushels, in barley 10.6 bushels, in spring wheat 5 bushels, and in winter wheat 6.5 bushels per acre more than small, plump seed of the same variety. Large, plump seed in oats proved more efficient by 7.9 bushels per acre than medium-sized plump seeds.

METHODS OF EXPERIMENTATION

The soil on which our plants were grown is classified by the United States Soil Survey as Hempstead silt loam. The rotation followed on the field where the plants were grown in 1914 and 1915 was as follows: Spring rye, clover, grain, corn with 14 tons of manure per acre, field peas, roots, and spring wheat. The soil is in a moderately high state of fertility. In 1916 and 1917 the plants were grown in a grain-clover-corn rotation, with 6 tons of manure applied preceding the corn. The soil is

not as productive as that on which the plants were grown in 1914 and 1915.

Data on rainfall and temperature are given in Table I. It is necessary to keep in mind the weather conditions during the growing season for each of the four years in order to interpret correctly the results of the work.

TABLE I.—*Normal rainfall and temperature, 1873–1903, with monthly deviations for the growing seasons 1914–1917, inclusive, Minneapolis, Minn.*

		Rainfall.					Temperature.				
	Year.	April.	May.	June.	July.	August.	April.	May.	June.	July.	August.
		Inches.	*Inches.*	*Inches.*	*Inches.*	*Inches.*	°*F.*	°*F.*	°*F.*	°*F.*	°*F.*
Normal	1873–1903	2.50	3.20	3.70	4.20	3.70	47.0	59.0	68.0	72.0	70.0
Deviation from normal.	1914	+1.25	−2.12	+4.62	−2.64	+5.01	−1.6	+2.9	− .8	+3.3	− .5
Do	1915	− .57	+ .06	+ .90	+2.11	− .20	+9.3	+5.2	−4.9	−4.6	−4.1
Do	1916	+ .63	+3.05	+ .53	−2.54	−2.03	−3.0	− .4	−4.3	+6.9	+2.4
Do	1917	− .74	+ .32	− .24	+ .25	− .86	−1.5	−2.7	−3.8	+ .9	−2.1

1914.—Seeds were planted on April 19 and some additional ones to make a more desirable number a few days later. Plants were harvested on August 4. With a temperature above normal for May and a rainfall 2 inches below the average, the plants made a luxuriant growth as to height, but produced only a moderate number of tillers. The abundant rainfall and approximately normal temperature of June were favorable for growth, which was checked prematurely by the high temperature and drought during early July. The latter part of July and early August were very wet and stemrust (caused by *Puccinia graminis tritici*) appeared on the plants when the kernels were in the milk stage. This resulted in a shriveling of practically all of the kernels.

1915.—Seeds were planted on April 19. Plants were harvested on August 17. The approximately normal rainfall for April and May, with the exceptionally favorable temperature, allowed the plants to make a luxuriant growth both as to height and tillering. Abundant rainfall during June and July with continued cool weather made conditions ideal for development in the late stages of growth. Stemrust was present in small amounts as the plants reached maturity, but did no damage that could be detected.

1916.—Seeds were planted on April 27. Plants were harvested on August 4. The rainfall was above normal for May and June, with the temperature approximately average during May and considerably below normal for June. In July the weather was dry and hot, conditions which hastened maturity and caused a moderate shriveling of some of the kernels.

1917.—Seeds were planted on April 11. Plants were harvested on July 31. Normal rainfall with continued cool weather up to July and approximately normal for that month made this a favorable year for wheat.

Marquis wheat, which belongs to the group *Triticum vulgare*, was used in the experiment throughout the 4-year period. This wheat was originated at the Central Experimental Farms, Ottawa, Canada, in 1892, by crossing Red Fife and an early-ripening wheat from India received in a sample of a commercial grade, Hard Red Calcutta, followed by a selection of individual plants in 1903. Marquis wheat is widely grown in the hard spring-wheat district in Canada and in the United States. A supply of the seed of this variety was obtained from Canada in 1913 and grown on University Farm that year. From the crop produced on University Farm in 1913, the individual seeds planted in 1914 and 1915 were selected. The seed planted in 1916 was selected from the 1915 crop. In 1917 the seed was taken from a Marquis line established by selecting individual plant 135 from the plants grown in 1914.

The seeds for planting were selected by hand and weighed to the fourth decimal place. If the fourth place was 5 or better, the figure in the third decimal place was increased by 1. As the seeds were weighed they were placed in coin envelopes. The seeds were then arranged in classes according to weight and consecutive numbers entered on the envelopes and at the same time on 3-inch wooden pot labels. The seeds were planted in 4-inch rows, 4 inches apart in the row, with the numbered pot label placed at the proper distance from each. One seed to each 16 square inches made the rate of seeding approximately 30 pounds per acre. For the years 1914 and 1915 the seeds were planted at approximately the same depth; in 1916 and 1917 all seeds were planted at precisely the same depth. For all the years except 1914, when a few additional seeds were planted later to make up the desirable number, all the seeds were planted on the same day. Before using the plants from the seeds sown later in 1914, comparison was made to ascertain whether they affected the results one way or another. Only where height at six weeks is involved was any effect found. Therefore, where height at six weeks is considered, the 219 plants from the first seeding are used. For all other characters, determinations were made on the full number of plants. In order to maintain uniform spacing for all plants, if a seed failed to grow, another plant of the same line was promptly taken from a reserve bed and substituted. These substitute plants were discarded at harvest. Border rows of the same variety were planted on all sides to obviate alley effect.

A few days before harvest, a dry-goods tag bearing the proper number was attached to each plant to identify it and to hold the culms together. All imperfect plants were discarded at this time. As each plant was

pulled, the upper portion was wrapped securely in paper to obviate any shattering. The plants were then hung in the laboratory to dry.

Data were taken on the seedlings and on the mature plants as shown in Table II. The whole plant was the unit used in making the determinations. The total weight of the plant was determined after the root had been severed at the surface of the ground and discarded. The weight of the seed was subtracted from the total weight of the plant for the straw weight determinations. Height of tallest culm was determined by measuring the primary stem from its attachment to the root to the tip of the apical spikelet. The average length of culms, including spikes, and of spikes only, per plant, was determined successively by laying them carefully end to end and taking their respective measurements. Then the total length divided by the number gave the average length of culms and spikes, respectively. Determinations were made on a total of 2,048 plants: 300 in 1914, 571 in 1915, 698 in 1916, and 479 in 1917. All determinations have been checked.

EFFECT OF ENVIRONMENT DURING GROWTH

In Table II are given the means, standard deviations, and coefficients of variability, with their respective probable errors, for each of the characters studied. The seed for the 1914 and 1915 planting was selected from the 1913 crop and for the two years had approximately equal mean weights and standard deviations. The seed planted in 1916 and 1917 was selected from the crop grown in 1915 and 1916, respectively. The mean weight of the seed planted in 1916 was 7.254±0.345 mgm. lower and that for 1917, 14.019±0.352 mgm. lower than the mean weight of the seed used the two previous years.[1]

TABLE II.—*Means, standard deviations, and coefficients of variability for the characters of the wheat studied*

Characters studied.	Means.			
	1914	1915	1916	1917
Weight of individual seeds planted, mgm.	32.580±0.393	33.033±0.260	25,779±0.226	19.014±0.237
Number of days from planting to second leaf	...	...	13.769± .049	26.373± .041
Height of plants at appearance of second leaf . . . cm.	6.401± .044	5.286± .039	5.351± .024	6.247± .251
Height of plants at six weeks . . . cm.	22.525± .150	23.277± .110	18.515± .076	15.638± .641
Height of tallest culm at maturity . . cm.	87.021± .389	113.663± .173	91.043± .149	98.763± .193
Average height of culms at maturity, cm.	68.833± .460	98.419± .272	84.693± .159	94.483± .208
Average length of spikes per plant . . cm.	7.638± .039	8.337± .029	7.985± .018	8.032± .020
Total length of culms per plant . . . cm.	248.000±4.483	686.821±5.220	225.043±1.740	268.966±2.026
Total length of spikes per plant . . . cm.	24.333± .423	54.461± .451	20.627± .165	23.601± .177
Number of culms per plant	3.606± .063	6.977± .053	2.651± .019	2.847± .020
Total yield per plant . . . dgm.	47.400±1.049	135.617±1.250	48.022± .391	66.558± .577
Yield of straw per plant . . . dgm.	39.600± .894	94.603± .832	30.644± .245	41.299± .357
Yield of kernels per plant . . . dgm.	8.153± .209	40.512± .466	17.305± .144	26.007± .227
Number of kernels per plant	55.860±1.081	142.629±1.428	69.183± .549	75.087± .648
Average weight of kernels per plant, mgm.	14.860± .156	27.563± .118	23.732± .056	34.050± .057

[1] The probable error of a difference is found by extracting the square root of the sum of the squares of the probable errors of the two numbers.

TABLE II.—*Means, standard deviations, and coefficients of variability for the characters of the wheat studied*—Continued

Characters studied.	Standard deviations.			
	1914	1915	1916	1917
Weight of individual seeds planted, mgm.	10.079±0.278	9.199±0.184	8.834±0.160	7.695±0.167
Number of days from planting to second leaf	....	....	1.928± .035	1.330± .029
Height of plants at appearance of second leaf ... cm.	1.120± .031	1.094± .022	.956± .017	.814± .017
Height of plants at six weeks ... cm.	3.282± .106	3.881± .078	2.972± .054	2.079± .045
Height of tallest culm at maturity ... cm.	8.527± .275	6.118± .122	5.837± .105	6.275± .136
Average height of culms at maturity, cm.	11.813± .325	9.638± .192	6.226± .112	6.779± .147
Average length of spikes per plant ... cm.	.996± .027	1.008± .021	.715± .012	.658± .014
Total length of culms per plant ... cm.	115.127±3.170	185.019±3.693	68.139±1.230	65.755± .143
Total length of spikes per plant ... cm.	10.861± .299	15.968± .319	6.473± .116	5.768± .125
Number of culms per plant	1.628± .044	1.862± .037	.766± .013	.652± .014
Total yield per plant ... dgm.	26.930± .742	44.137± .881	15.320± .276	18.748± .408
Yield of straw per plant ... dgm.	22.957± .632	29.466± .588	9.601± .173	11.584± .252
Yield of kernels per plant ... dgm.	5.378± .148	16.498± .329	5.674± .102	7.383± .160
Number of kernels per plant	27.753± .764	50.584±1.001	21.514± .388	21.041± .458
Average weight of kernels per plant, mgm.	3.999± .110	4.185± .084	2.211± .039	1.874± .040

Characters studied.	Coefficients of variability.			
Weight of individual seeds planted, mgm.	30.940±0.930	27.850±0.597	34.27±0.69	40.47±1.02
Number of days from planting to second leaf	....	....	14.01± .26	5.05± .11
Height of plants at appearance of second leaf ... cm.	17.500± .497	20.710± .431	17.88± .33	13.04± .29
Height of plants at six weeks ... cm.	14.570± .479	16.670± .342	16.05± .30	13.29± .29
Height of tallest culm at maturity ... cm.	9.799± .316	5.380± .107	6.41± .12	6.35± .14
Average height of culms at maturity, cm.	17.160± .486	9.790± .195	7.35± .13	7.18± .16
Average length of spikes per plant ... cm.	13.050± .366	12.090± .245	8.96± .16	8.19± .18
Total length of culms per plant ... cm.	46.420±1.529	26.940± .575	30.28± .59	24.45± .56
Total length of spikes per plant ... cm.	44.640±1.450	29.320± .634	31.36± .62	24.44± .56
Number of culms per plant	45.150±1.475	26.690± .569	28.92± .56	22.91± .52
Total yield per plant ... dgm.	56.810±2.007	32.550± .715	31.90± .63	28.17± .66
Yield of straw per plant ... dgm.	57.970±2.064	31.150± .679	31.33± .62	28.05± .66
Yield of kernels per plant ... dgm.	65.950±2.484	40.720± .938	32.79± .65	28.39± .67
Number of kernels per plant	49.680±1.672	35.470± .792	31.10± .61	28.02± .66
Average weight of kernels per plant, mgm.	37.160±1.156	15.180± .310	9.32± .17	5.50± .12

The somewhat higher productivity of the soil on which the plants were grown in 1914 and 1915, the conditions favoring or retarding growth each season throughout the four years, and the mean weight of the seed planted are very pertinent to the consideration of the variability of the plant characters.

MEANS

The mean for number of days from planting to second leaf was determined for two years only. The greater number of days from planting to second leaf in 1917 as compared with 1916 was due to the lower temperature and drier weather during the period between planting of the seed and emergence in the former year. The means for height in centimeters at appearance of second leaf are 5.286 ± 0.039 for 1915, 5.351 ± 0.024 for 1916, 6.247 ± 0.251 for 1917, and 6.401 ± 0.044 for 1914, with no significant difference between the first two or the last two. One of the highest and

one of the lowest means for height at second leaf was for plants grown on the more productive soil; and likewise one of the highest and one of the lowest for plants grown from the greatest mean weight of seed. Temperature and moisture conditions appear to have had a large influence in rate of development at this early stage.

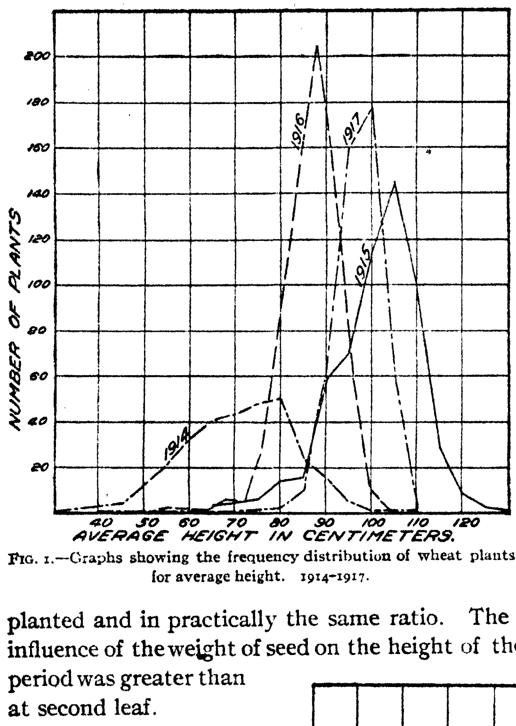

FIG. 1.—Graphs showing the frequency distribution of wheat plants for average height. 1914–1917.

The means in centimeters for height at six weeks are 22.525 ± 0.150 in 1914, 23.277 ± 0.110 in 1915, 18.515 ± 0.076 in 1916, and 15.638 ± 0.641 in 1917, with no significant diference between the first two. This is in the same order as the means for weight of seed planted and in practically the same ratio. The indications are that the influence of the weight of seed on the height of the plants at the six weeks' period was greater than at second leaf.

The means for height of tallest culm at maturity in ascending order of magnitude are 87.021 ± 0.389 for 1914, 91.043 ± 0.149 for 1916, 98.763 ± 0.193 for 1917, and 113.663 ± 0.173 for 1915.

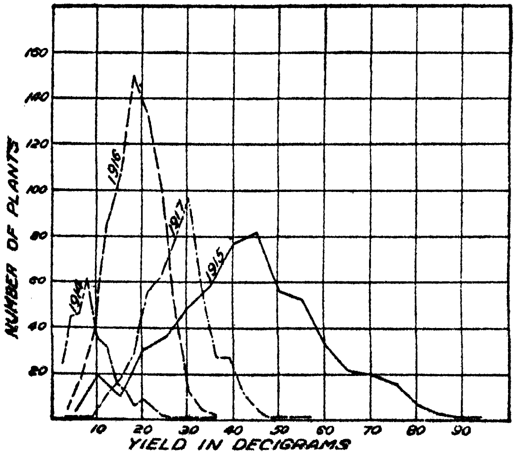

FIG. 2.—Graphs showing the frequency distribution of wheat plants for yield of kernels. 1914–1917.

The means for average height of culms at maturity, average length of spikes, number of kernels, yield of kernels, and total yield per plant follow the same order as those for height of plant. The sequence for average height of culms and yield of kernels per plant representing the

order for this group of characters is shown in the frequency distribution graphs (fig. 1, 2). For each of this group of characters the lowest mean occurs in 1914, the season least favorable to normal development during the latter part of the growing season, and the means for the other three years occur in ascending order according to the favorableness as a whole of the growing season for wheat, 1916, 1917, and 1915, respectively.

The means for number of culms per plant are 2.651 ±0.019 in 1916, 2.847±0.020 in 1917, 3.606±0.063 in 1914, and 6.977±0.053 in 1915. The means for yield of straw, total length of culms, and total length of spikes per plant are in practically the same order as those for number of culms.

For number of culms per plant representing this group of characters, the order of the means is shown in the frequency distribution graph (fig. 3). The magnitude of the means for this group of characters is largely dependent on the favorableness of conditions for growth during the early part of the season. This order is 1916, 1917, 1914, and 1915, respectively.

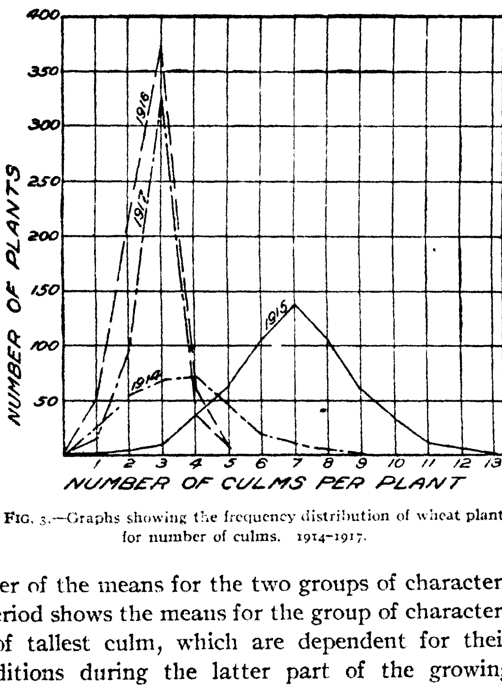

FIG. 3.—Graphs showing the frequency distribution of wheat plants for number of culms. 1914–1917.

Comparison of the order of the means for the two groups of characters throughout the 4-year period shows the means for the group of characters represented by height of tallest culm, which are dependent for their development upon conditions during the latter part of the growing season, follow the order of optimum conditions during that time; and that the means for the other group of characters represented by number of culms, which develop largely during the early part of the season, follow the order of the best conditions for early growth.

The means for average weight of kernel are 14.860±0.156 in 1914, 23.732±0.056 in 1916, 27.563±0.118 in 1915, and 34.050±0.057 in 1917. The frequency distribution graph (fig. 4) shows this order. In 1914 and 1916, the sequence of the means for average weight of kernels is the same as yield of grain and number of kernels; but in 1915 and 1917 in the reverse order. The kernels of the 1914 crop were shriveled, as were also some of those of the 1916 crop. In 1915 and 1917 all kernels were well filled. Under the especially favorable conditions which prevailed

throughout the entire growing season in 1915 a larger number and greater yield of kernels was produced, but the average weight of the kernels was not as great as in 1916, when conditions favored a more normal development.

As indicated by the means, the various characters studied responded more or less directly to external conditions which prevailed while each was making its most rapid development. The number of culms per plant and the yield of straw were influenced most by environment during the early part of the growing season, and number, yield, and average weight of kernels by environment during the latter part of the growing season.

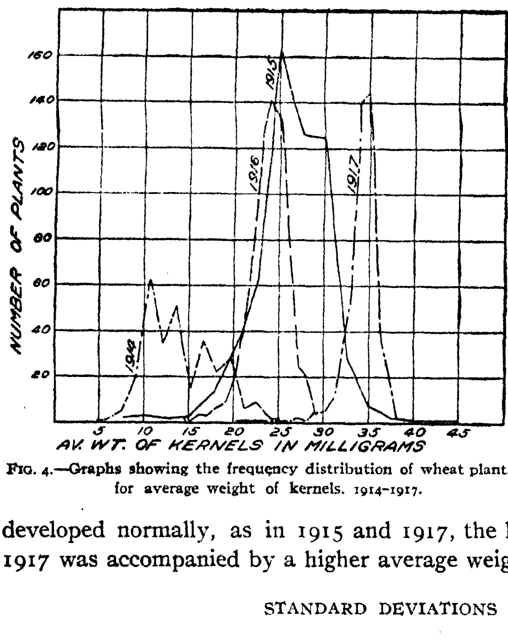

FIG. 4.—Graphs showing the frequency distribution of wheat plants for average weight of kernels. 1914–1917.

When growth was retarded or stopped by environmental conditions, lower yields of straw resulted from a reduction in the number, total, or average length of culms per plant; and lower yields of grain, from a reduction in the number of kernels; and for 1914 and 1916 only, a lower average weight of kernels. The kernels were more or less shriveled in 1914 and 1916. When the grain developed normally, as in 1915 and 1917, the lower yield of kernels in 1917 was accompanied by a higher average weight per kernel.

STANDARD DEVIATIONS

The standard deviations for number of days from planting to second leaf are 1.928 ± 0.035 in 1916 and 1.330 ± 0.029 in 1917.

For height at second leaf the two highest means are accompanied by the highest and lowest standard deviations. Better root development in 1917 during the prolonged cool period intervening between the time of planting the seed and emergence, which may have permitted the plants to begin growth at the surface more nearly at the same time, is a possible explanation of the lower standard deviation accompanying the higher mean.

The standard deviations for height at six weeks are in the same order as the means, with significant differences between any two except those in 1914 and 1916. The variability of average height at second leaf and at six weeks, measured by the standard deviations, is comparatively low during the 4-year period.

For height of tallest culm, average height of culms, and average length of spikes the means in 1914 are lower, but the standard deviations are as high or higher than the standard deviations for these characters in any of the three other years. The comparatively high variability in 1914 of average height of culms is indicated on the frequency distribution graph (fig. 1). The differences in centimeters between the height of tallest culm and average length of culms are 18.188 ± 0.602 in 1914, 15.214 ± 0.322 in 1915, 6.350 ± 0.218 in 1916, and 4.280 ± 0.284 in 1917. The greater difference in height between the tallest culm and the average of the culms in 1914 indicates that the drouth in early July and the stemrust in late July and early August of that year prevented the secondary culms from approaching in height the main culm as closely as they did the three other years. This would tend to increase the variability of these two characters as well as that of average length of spikes. After making due allowance for the abnormal conditions in 1914, it is of interest to note the comparatively low variability of height of tallest culm as indicated by the standard deviations.

For number and average weight of kernels and total yield of plants the means were lowest in 1914, but the standard deviations for these characters in the same year were either next to the highest or equal to the highest.

The mean for yield of kernels in 1914 was reduced materially, owing to the drought and black stemrust, and the standard deviation is also low.

The mean for average weight of kernels per plant was highest in 1917, but it is accompanied by the lowest standard deviation in the 4-year period. This is indicated on the frequency distribution graph (fig. 4).

For number of culms, total length of culms, total length of spikes, and weight of straw per plant the means for 1914 were either almost equaled or exceeded by those for 1915 and 1916, more favorable years; but the standard deviations are equaled or exceeded only by those for the 1915 crop.

In general, the standard deviations tend to follow the same order as the means, the variability being greatest where the means are the greatest, due in both instances to favorable conditions for development. Exceptions to this tendency may be due in part to the frequent smaller differences between standard deviations as judged by their probable errors compared with the differences between means as judged by their probable errors. Average weight of kernel had the highest mean in 1917 accompanied by the lowest standard deviation, which is an exception. A number of exceptions occurred in 1914, owing to the very favorable condition for development during the first part and the opposite conditions during the latter part of the growing season.

COEFFICIENTS OF VARIABILITY

With few exceptions the coefficients were higher in 1914 than in the three other years. This corresponds to the generally lower means for that year.

As is indicated by the coefficients, number of days to second leaf and average weight of kernels in 1917 varied least, but each character was highly variable from year to year. Height at six weeks, height at maturity, average height of culms, and average length of spikes were comparatively low in variability each year and from year to year. This confirms similar indications by the standard deviations.

As indicated by the coefficients of variability, the greatest variation in the 4-year period occurred in 1914 for total weight per plant, yield of of straw, and yield of kernels.

CORRELATIONS

Correlation coefficients were determined for weight of seed used and each of the resultant plant characters listed in Table III. The assembled data also offered the opportunity to study the interrelation of plant characters for which the coefficients of correlation are presented in Table XIII. Since it was not considered feasible to present all the correlation tables, the selection for presentation was confined to those likely to be of most value and interest. (See Tables IV–XI; XIV–XXI.)

TABLE III.—*Coefficients of correlation between weight of seed and characters of the resultant plants*

Characters studied.	1914	1915	1916	1917
Number of days from planting to second leaf	....	....	−0.484±0.019	−0.634±0.018
Height of plants at appearance of second leaf ... cm	0.146±0.038	0.114±0.027	.169± .024	.259± .028
Height of plants at six weeks ... cm	.356± .040	.445± .022	.649± .014	.712± .015
Height of tallest culm at maturity ... cm	.196± .037	− .037± .028	.311± .023	.074± .030
Average height of culms at maturity ... cm	.093± .038	− .099± .028	.192± .024	.118± .030
Average length of spikes per plant ... cm	.007± .038	− .193± .027	.126± .025	.202± .029
Total length of culms per plant ... cm	.251± .036	.066± .028	.460± .020	.395± .026
Total length of spikes per plant ... cm	.259± .036	− .018± .028	.442± .020	.417± .025
Number of culms per plant	.232± .036	.116± .027	.420± .021	.398± .025
Total weight per plant ... dgm	.229± .036	.064± .028	.423± .021	.435± .024
Yield of straw per plant ... dgm	.226± .036	.046± .028	.407± .021	.401± .025
Yield of kernels per plant ... dgm	.143± .038	.088± .028	.445± .020	.478± .024
Number of kernels per plant	.246± .036	.076± .028	.458± .020	.465± .023
Average weight of kernels per plant, mgm	− .062± .038	.086± .028	.055± .025	.141± .030

TABLE IV.—*Weight in milligrams of individual seeds planted correlated with height in centimeters of plant at six weeks. 1914*

Height of plant at six weeks (cm.).	Weight of individual seeds planted (mgm.).										Frequency.
	13	15	17	19	21	23	25	27	29	31	
18		1			3	1					5
20	1		2	4	3	2					12
22		2		4	4	4	1				15
24			2	5	5	5	2		1		20
26			1	1	7	5	5	1	1		21
28			3	2	4	4					13
30			1	1		2	2				6
32				1	1	2	2	1			7
34					2	4	2	1			9
36					2	9	2	1			14
38			1	1	3	2	2	1	4	1	15
40				3	6	6	3	2	3		23
42		1		2	1	9	4	1	1		19
44				3	4	2	6	4	1		20
46		1	1	1	1	6	1	1	2	1	15
48						1		1			2
50							1				1
52					1		1				2
Frequency	1	5	11	28	47	64	34	14	13	2	219

Correlation = 0.356 ± 0.040.

TABLE V.—*Weight in milligrams of individual seeds planted correlated with height in centimeters of plant at six weeks. 1915*

Height of plant at six weeks (cm.).	Weight of individual seeds planted (mgm.).														Frequency.
	9	11	13	15	17	19	21	23	25	27	29	31	33	35	
16				1	4		1	1							7
18			1	1	3	2	1								8
20				3	6	7	8	6	5						35
22	1		1	1	7	9	13	15	5						52
24			1		8	4	17	25	6	3					64
26						4	8	10	14	8	1				45
28				1		3	4		2	1					11
30			1			2	6	1	8	1	1				20
32					6	1	4	15	15	6					47
34				1	2	2	4	7	10	8	3	1			38
36						1	2	6	6	4	2				21
38					1			4	3		2				10
40				1		4	10	6	10	9	6				46
42			1		1	6	4	7	12	15	14	5			65
44					1	2		4	7	12	19	2		2	49
46			1		4	2	2	6	9	9	1	1			35
48						1	1	2	3	1	2				10
50				1	1	1	2			2	1				8
Frequency	1		6	10	44	51	87	113	115	79	52	9		2	571

Correlation = 0.445 ± 0.022.

TABLE VI.—*Weight in milligrams of individual seeds planted correlated with height in centimeters of plant at six weeks. 1916*

Height of plant at six weeks (cm.).	Weight of individual seeds planted (mgm.).																			Frequency.
	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
8			1	2	1															4
10	1			2	4	2	4	2												15
12				1	7	9	6	2	3		1									29
14		1		1	5	5	13	11	7	3	2									48
16					4	5	8	16	7	9	6	3		1						59
18			1		1	1	4	8	4	6	9		1	1						36
20							2	2	7	4	6	2	1	1						25
22				1	2		2	6	5	11	6	2	3							38
24							3	7	5	9	21	6	13	2	2					68
26						1	3	6	6	11	20	17	7	5	1					77
28					1	2		3	4	5	11	13	9	9	4	1				62
30		1				3			3	2	12	9	9	2	2		1			44
32		1				1	1				1	4	3	1	2	2	1			17
34							1	2	3	4	3	7	5	3	1		2		1	32
36						1			1	3	3	5	10	8	9	4	1			45
38					1		2	1				11	11	8	13					47
40								1	2	1	2	4	7	6	4	3				30
42					1		1	1	1		1	2	3	3	3					16
44								1				1		2	1	1				6
Frequency	1	3	2	7	27	30	50	69	58	68	104	86	82	52	42	11	5	...	1	698

Correlation = 0.649 ± 0.015.

TABLE VII.—*Weight in milligrams of individual seeds planted correlated with height in centimeters of plant at six weeks. 1917*

Height of plant at six weeks (cm.).	Weight of individual seeds planted (mgm.).															Frequency.
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
6	1				2	4	8	8	2							25
8					1	1	8	10	9	3						32
10							8	6	14	9	2	2				41
12								2	11	14	5					32
14					1			2	7	8	15	1				34
16							1	2	3	8	21	4				39
18					1					9	15	7	1			33
20									2	8	13	11	5			39
22								2	4	4	8	9	9	1		37
24							1	2	1	4	10	2	12	4		36
26							1		1	3	4	11	12	6		38
28						1				1	7	11	12	3	1	36
30				1					1	2	5	9	6	7	1	32
32											3	6	10	5	1	25
Frequency	1	...	...	1	5	6	27	34	55	73	108	73	67	26	3	479

Correlation = 0.712 ± 0.015.

TABLE VIII.—*Weight in milligrams of individual seeds planted correlated with yield in decigrams of kernels per plant. 1914*

Yield of kernels per plant (dgm.).	Weight of individual seeds planted (mgm.). 1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	Frequency.
14		1	1		1														3
16		1		1	2														4
18	7	3	5	3	1		1												20
20	4	5	3	5		1	2	2		1									23
22		3	9	6	3	5	1					1							28
24	2	5	2	8		5	1	1	1	1	1								27
26		4	2	2	2	1	3	1											15
28	1	1	2	2					1										7
30		1		2	3	1			1										8
32				3	1		1			1									6
34	1	1	2	1		4		1	1										11
36			2	2	2	4		1	1	2									14
38	1	3	1	4		2	1	2			3		1						18
40		7	2	6	4	2	2	1	1	2	1	1			1			1	31
42	3	2	4	6	6	3	3	2		1									30
44	2	4	8	5	4		2				1								26
46	1	3	2	3	4	4		2		1									20
48	2				2		1												5
50		1	1	2															4
Frequency	24	45	46	61	35	32	18	13	6	9	6	2	1		1			1	300

Correlation = 0.143 ± 0.038.

TABLE IX.—*Weight in milligrams of individual seeds planted correlated with yield in decigrams of kernels per plant. 1915*

Yield of kernels per plant (dgm.)	Weight of individual seeds planted (mgm.) 2.5	7.5	12.5	17.5	22.5	27.5	32.5	37.5	42.5	47.5	52.5	57.5	62.5	67.5	72.5	77.5	82.5	87.5	92.5	97.5	Frequency.
16	1	1				1		1	2			1									7
18		1			1	1	2		2		1										8
20		2	1	1	3	6	4	4	2	2	7		2		1						35
22	2		1	1	4	2	5	7	4	7	6	7	2	2	1	1					52
24		4	1	2	3	8	8	5	8	10	7	2	4	2							64
26			1			3	5	7	9	4	7	3		3	1		1		1		45
28			1	1	1	1	2	3	1					1							11
30		1		1	3	3	2	1	2	2	3		1				1				20
32		1	2	3		2	5	13	8	5	3	3	1	1							47
34		1		3	4	6	5	4	7	5	1		1	1							38
36					2	4	2	2	5	1	2	2	1								21
38		1			1		1	3	1			1			1	1					10
40		1		5	3	4	4	7	11	1	3	1	2	3	1						46
42		4	1	6	7	6	4	9	6	4	7	3	2	2	3					1	65
44		1	1	1	1		5	7	9	7	3	4	5	3	1	1					49
46		1	1	5	3	2	2	2	5	7	1	3			3						35
48							1	1		1	1	1			2	2		1			10
50				1			1	1				1		1	1	1				1	8
Frequency	3	19	10	30	36	49	58	77	82	56	52	32	21	19	15	6	2	1	1	2	571

Correlation = 0.088 ± 0.028.

TABLE X.—*Weight in milligrams of individual seeds planted correlated with yield in decigrams of kernels per plant. 1916*

Yield of kernels per plant (dgm.)	Weight of individual seeds planted (mgm.) 2.25	3.75	5.25	6.75	8.25	9.15	11.25	12.75	14.25	15.75	17.25	18.75	20.25	21.75	23.25	24.75	26.25	27.75	29.25	30.75	32.25	33.75	Frequency.
8	...	...	1	...	1	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4
10	1	...	...	...	4	2	1	2	2	2	1	...	...	...	...	...	...	...	...	...	...	...	15
12	...	...	3	1	2	7	3	4	5	1	...	1	1	1	...	...	...	...	...	...	...	...	29
14	...	...	1	1	5	4	12	7	5	6	2	3	1	...	1	...	...	...	...	...	...	...	48
16	...	1	2	2	1	6	5	4	6	8	8	8	4	1	2	...	...	1	...	...	...	...	59
18	...	...	...	1	1	3	2	2	7	4	4	4	6	...	1	...	...	1	...	...	...	...	36
20	...	...	...	...	1	...	...	4	2	6	6	2	3	1	...	...	...	...	...	...	...	...	25
22	...	1	...	...	1	1	4	3	4	3	5	3	6	1	2	2	...	...	...	...	...	...	38
24	...	...	...	1	...	...	2	5	9	6	7	10	4	6	5	7	3	2	1	...	...	...	68
26	...	...	1	1	1	6	3	3	5	7	11	6	8	12	4	7	1	1	...	...	...	...	77
28	1	1	...	...	1	1	4	2	4	7	5	2	10	12	2	8	...	2	...	...	...	...	62
30	1	...	1	...	2	1	1	5	4	3	7	10	2	1	5	1	...	...	...	...	...	...	44
32	1	...	...	1	...	1	...	...	...	2	3	3	2	1	1	2	...	...	...	...	...	...	17
34	...	...	...	...	...	...	2	1	...	2	2	6	3	5	3	3	2	...	...	1	1	1	32
36	...	...	...	...	...	1	2	2	3	2	2	9	2	7	6	4	1	4	...	...	...	...	45
38	...	...	1	...	1	...	1	...	...	2	6	8	7	3	6	2	6	2	1	...	1	...	47
40	...	1	...	...	...	2	1	...	1	1	3	2	1	4	5	1	5	...	1	1	...	1	30
42	...	1	...	2	...	...	1	1	...	...	1	...	1	3	...	3	1	1	...	1	...	...	16
44	...	...	...	...	...	...	...	...	...	...	...	1	2	1	1	...	...	...	...	1	...	...	6
Frequency	4	5	10	12	21	36	44	46	57	62	73	78	63	59	44	40	19	14	3	4	2	2	698

Correlation = 0.445 ± 0.020.

TABLE XI.—*Weight in milligrams of individual seeds planted correlated with yield in decigrams of kernels per plant. 1917*

Yield of kernels per plant (dgm.)	Weight of individual seeds planted (mgm.) 1.5	8.5	7.5	10.5	13.5	16.5	19.5	22.5	25.5	28.5	31.5	34.5	37.5	40.5	43.5	46.5	49.5	52.5	55.5	Frequency.
6	1	...	...	3	7	4	6	2	1	1	...	...	...	...	...	...	...	...	...	25
8	1	...	1	6	2	2	8	6	3	3	...	...	...	...	...	...	...	...	...	32
10	...	...	...	...	3	8	9	7	7	4	3	...	...	...	...	...	...	...	...	41
12	...	...	...	...	...	7	4	4	10	2	2	2	1	...	...	...	...	...	...	32
14	...	...	...	1	1	2	2	6	5	9	5	...	1	2	...	...	...	...	...	34
16	...	...	...	1	1	...	4	3	8	5	8	5	3	1	...	...	...	...	...	39
18	...	...	...	...	...	...	4	2	5	13	4	1	4	...	...	...	...	...	...	33
20	...	...	...	...	...	1	6	4	9	9	8	1	1	...	...	...	...	...	...	39
22	...	...	...	...	...	...	1	8	10	9	4	1	1	2	1	...	...	...	...	37
24	...	...	...	...	2	1	3	8	4	9	6	1	2	...	...	...	...	...	...	36
26	...	...	...	...	2	1	3	6	4	11	7	2	1	1	...	...	...	...	...	38
28	...	...	...	...	...	2	4	3	3	8	6	5	3	1	...	...	1	...	...	36
30	...	1	...	...	...	...	1	3	4	5	4	6	4	2	1	...	...	...	1	32
32	...	...	...	...	...	1	...	...	5	5	1	3	6	2	1	1	...	...	...	25
Frequency	2	1	1	11	18	29	55	62	78	93	58	27	27	11	3	1	1	...	1	479

Correlation = 0.478 ± 0.023.

RELATION OF WEIGHT OF SEED USED TO THE RESULTANT PLANT CHARACTERS

Inspection of the coefficients given in Table III shows that, with certain exceptions, the correlation coefficients in 1914 and 1915 are lower than those for the same characters in 1916 and 1917.

The relatively low correlation in 1914 and 1915 corresponds to the comparatively high variability in these two years, both of which are due in part to (a) the extreme climatological and pathological conditions, (b)

the somewhat higher productivity of the soil, and (c) the higher mean and greater range in weight of seed planted.

Correlation coefficients for number of days from planting to second leaf were determined in 1916 and 1917 only. The correlation coefficients are relatively high, indicating that the plants from the heavier seeds reach the second leaf stage sooner than the plants from the lighter seeds. Correlation is highest in 1917, the year in which the mean weight of the seed was the lowest.

Weight of seed correlated with height of plants at second leaf gave coefficients varying from 0.114±0.027 in 1915 to 0.259±0.028 in 1917 and correlated with height at six weeks, a variation from 0.356±0.040 in 1914 to 0.712±0.015 in 1917. In each of the four years the coefficients as compared with their probable errors show a fair correlation for height at second leaf and a considerably higher correlation for height at six weeks. This indicates that at the appearance of the second leaf the greater food supply available to the plants from the larger seeds had not yet exerted its influence. An extreme difference of 0.145±0.038 in the coefficients for height at second leaf and 0.356±0.042 for height at six weeks during the 4-year period shows that correlation between weight of seed and both of the characters was influenced considerably by environment.

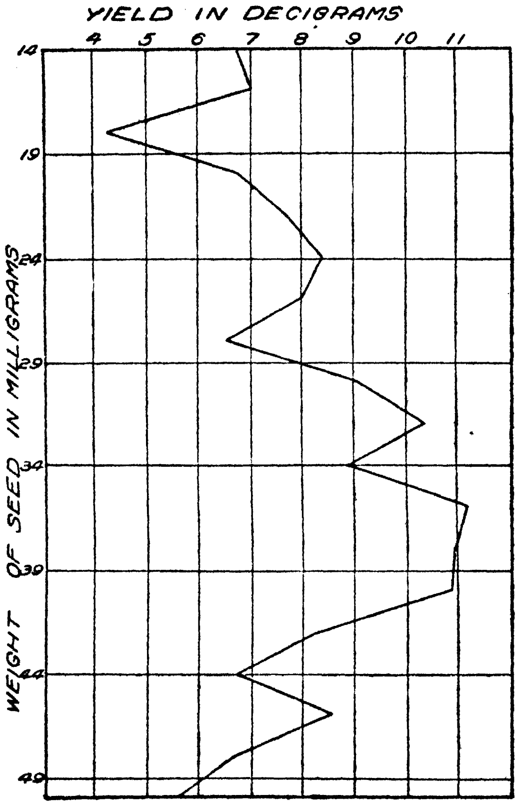

Fig. 5.—Graph showing regression for weight of seed and yield of kernels per wheat plant in 1914.

Between weight of seed and height of tallest culm at maturity the correlation coefficients are −0.037±0.028 in 1915, 0.074±0.030 in 1917, 0.196±0.037 in 1914, and 0.311±0.023 in 1916. As indicated by the

coefficients in terms of their probable errors, there was practically no correlation between weight of seed and height of tallest culm at maturity in 1915 and 1917 and a good correlation in 1914 and 1916. The coefficients of correlation between weight of seed and average height at maturity are 0.093 ± 0.038 in 1914, −0.099 ± 0.028 in 1915, 0.118 ± 0.030 in 1917, and 0.192 ± 0.024 in 1916. The coefficients of correlation of the two characters range from 2.45 times the probable error in 1914 to 8 times the probable error in 1916.

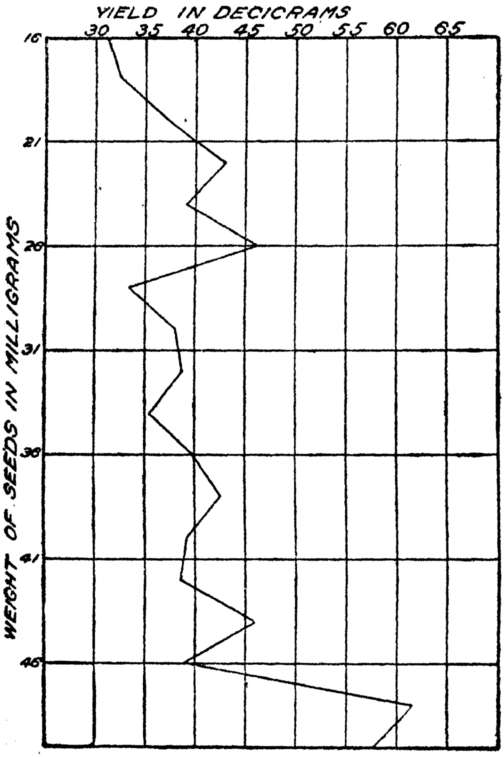

FIG. 6.—Graph showing regression for weight of seed and yield of kernels per wheat plant in 1915.

Between weight of seed planted and average weight of kernels harvested the coefficients are −0.062 ± 0.038 in 1914, 0.055 ± 0.025 in 1916, 0.086 ± 0.028 in 1915, and 0.141 ± 0.030 in 1917. While the coefficients are low in each of the four years, a slight correlation is indicated in 1914 and 1916, and in 1915 the coefficient is three times and in 1917 4.7 times their respective probable errors. The most significant correlation between the two characters occurred in 1917 when the seeds planted were selected from a line established through the selection of individual plant 135 of the 1914 crop. Since the results indicate that the Marquis wheat used in this experiment was not homozygous for weight of seed, it can not be considered a pure line for this character.

The coefficients for weight of seed planted correlated with yield of kernels per plant are 0.088 ± 0.028 in 1915, 0.143 ± 0.038 in 1914, 0.445 ± 0.020 in 1916, and 0.478 ± 0.023 in 1917. In 1914 and 1915 the coefficients of correlation are low, 3.1 times and 3.8 times their probable errors, respectively, with no significant difference between them. The coefficients in 1916 and 1917 are considerably greater than those in 1914 and 1915.

The weight classes for the individual seeds planted, number of plants in each class harvested, and the average yield, in decigrams, of kernels per plant are given in Table XII. This gives the same data with respect to yield from kernels of different weights as is given in the correlation tables but in more direct form. That increase in weight of seed planted was not consistently followed by increased yield of kernels is evident.

TABLE XII.—*Weight classes of seeds planted and average yield of kernels per plant*

Weight classes of individual seeds planted.	1914		1915		1916		1917	
	Number of plants harvested.	Average yield per plant.	Number of plants harvested.	Average yield per plant.	Number of plants harvested.	Average yield per plant.	Number of plants harvested.	Average yield per plant.
Mgm.		*Dgm.*		*Dgm.*		*Dgm.*		*Dgm.*
6	....	....	....	....	....	....	25	16.38
8	....	....	....	....	4	9.00	32	18.28
10	....	....	....	....	15	11.25	41	21.77
12	....	....	....	....	29	11.72	32	23.91
14	3	5.67	....	....	48	12.84	34	26.21
16	4	7.00	7	31.07	59	14.89	39	28.01
18	20	4.20	8	32.50	36	15.88	33	28.23
20	23	6.74	35	37.21	25	16.23	39	26.50
22	28	7.64	52	42.98	38	15.91	37	27.93
24	27	8.26	64	38.98	68	18.95	36	26.08
26	15	7.93	45	46.28	77	17.89	38	26.84
28	7	6.43	11	33.36	62	18.52	36	29.08
30	8	9.00	20	38.00	44	16.47	32	31.22
32	6	10.33	47	38.88	17	17.25	25	32.70
34	11	8.82	38	35.39	32	21.14	....	....
36	14	11.14	21	39.88	45	20.08	....	....
38	18	10.89	10	42.50	47	20.86	....	....
40	31	10.81	46	39.02	30	20.90	....	....
42	30	8.20	65	38.42	16	18.94	....	....
44	26	6.62	49	45.97	6	22.50	....	....
46	20	8.50	35	38.79	....	....	....	....
48	5	6.60	10	61.50	....	....	....	....
50	4	5.50	8	57.50	....	....	....	....

Regression for yield of kernels in each of the four years is shown in figures 5, 6, 7, and 8. Regression for yield was consistently greater in 1916 (fig. 7) for the seeds up to 24 mgm. than for the larger seeds. Apparently increase in amount of endosperm in 1916 and 1917 up to the weights of seeds indicated gave more uniformly proportionate increases in yield than were given by increases in endosperm beyond these amounts.

Very similar to the correlations between weight of seeds and yield of kernels in each of the four years are those between weight of seed and total length of culms, total length of spikes, number of culms, total yield, yield of straw and number of kernels per plant.

The correlation between weight of seed and plant characters at maturity in 1916 and 1917 was, for height of tallest culm, low and variable; for average height of culms and average length of spikes, low but less variable; and for total length of culms, total length of spikes, number of culms, total weight, and yield of grain and straw, medium, with little fluctuation. The only significant difference in 1916 and 1917 between coefficients for weight of seed planted and characters at maturity is

that between those for height of tallest culm. Therefore it is evident that on soil of medium productivity, the more favorable weather conditions in 1917 as compared with that in 1916 did not influence correlation to any marked extent.

In 1914 and 1915 the coefficients for all plant characters at maturity are comparatively low; and, with the exception of those for number of culms, yield of kernels, and average weight of kernels, they are significantly lower in 1915 than in 1914. This difference is due to the highly favorable environmental conditions in 1915.

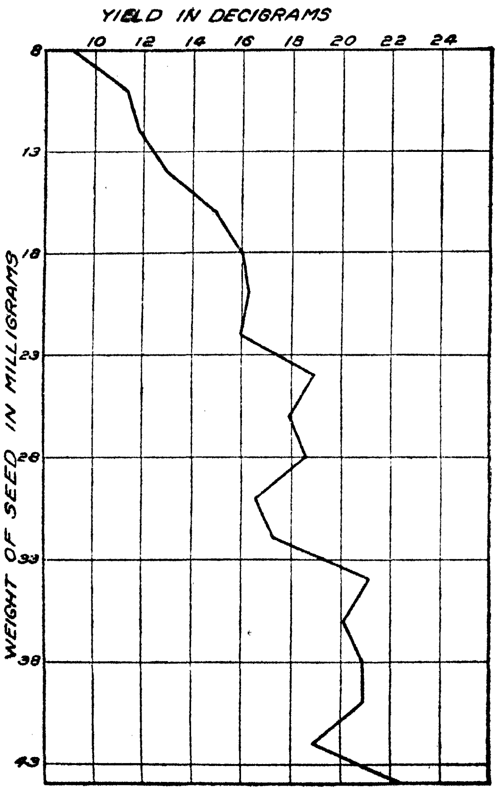

FIG. 7.—Graph showing regression for weight of seed and yield of kernels per wheat plant in 1916.

Considered as a whole, there is a distinct tendency toward correlation between weight of seed sown and the characters of the resultant plants. However, the correlation, even under average conditions, is not high in any instance, and is subject to the influence of environmental conditions to so marked an extent that with some characters the relation may be obliterated entirely; and with other characters, including yield, may be made so slight that under ordinary conditions of experiment no relation could be detected.

INTERRELATION OF PLANT CHARACTERS

The coefficients given in Table XIII show that only in the group where yield of kernels is correlated with other characters is there a general tendency toward less correlation in 1914 and 1915 than in 1916 and 1917. This condition although much less marked, is similar to that found when weight of seed was correlated with plant characters at maturity and is

due to the differences in environment between the first two and last two years.

TABLE XIII.—*Coefficients of correlations between plant characters*

Characters studied.	1914	1915	1916	1917
Yield of kernels per plant (dgm.) and—				
Number of kernels per plant	0.851±0.010	0.881±0.006	0.952±0.002	0.973±0.001
Average weight of kernels per plant . mgm.	.550± .027	.504± .021	.370± .022	.306± .027
Number of culms per plant	.500± .029	.669± .015	.818± .008	.824± .009
Average height of culms per plant . cm.	.384± .033	.303± .025	.478± .019	.452± .024
Average length of spikes per plant . cm.	.357± .034	.344± .024	.459± .020	.591± .020
Total length of spikes per plant . cm.	.636± .023	.808± .009	.910± .004	.911± .005
Number of culms per plant and—				
Average length of spikes per plant . cm.	.061± .038	.024± .028	.039± .025	.236± .029
Total length of spikes per plant . cm.	.872± .009	.839± .008	.958± .002	.946± .003
Average weight of kernels per plant (mgm.) and—				
Number of kernels per plant	.137± .038	.192± .027	.160± .024	.160± .030
Number of culms per plant	.071± .038	.137± .027	−.054± .025	−.009± .030
Average length of spikes per plant . cm.	.153± .038	.120± .027	.552± .017	.411± .025
Total length of spikes per plant . cm.	.001± .038	.159± .027	.079± .025	.091± .030
Average height of culms per plant (cm.) and—				
Number of kernels per plant	.257± .036	.339± .025	.364± .022	.431± .025
Average weight of kernels per plant . mgm.	.458± .030	.071± .028	.648± .014	.426± .025
Number of culms per plant	.195± .037	.092± .028	.046± .025	.205± .029
Average length of spikes per plant . cm.	.315± .035	.419± .023	.775± .010	.668± .017
Total length of spikes per plant . cm.	.036± .038	.260± .026	.235± .024	.351± .027
Height of plants at appearance of second leaf (cm.) and—				
Height of plants at six weeks . cm.	.406± .038	.467± .022	.470± .019	.466± .024
Height of tallest culm at maturity . cm.	.380± .033	.270± .026	.272± .023	.211± .029
Height of plants at six weeks (cm.) and—				
Height of tallest culm at maturity . cm.	.399± .038	.236± .026	.523± .018	.314± .027

TABLE XIV.—*Yield in decigrams of kernels per plant correlated with average weight in milligrams of kernels per plant. 1914*

Average weight of kernels per plant (mgm.).	Yield of kernels per plant (dgm.): 5.25	6.75	8.25	9.75	11.25	12.75	14.25	15.75	17.25	18.75	20.25	21.75	23.25	24.75	26.25	Frequency.
1		2	1	2	8		7		2	1	1					24
3	1		1	7	18	5	5	1	5	1	1					45
5			3	4	11	8	10	2	3	1	4					46
7			1	6	10	9	12	6	7	4	3		2		1	61
9				2	10	7	5	1	5	1	4					35
11					4	2	6	3	6	3	5	1	2			32
13					1	2	3	2	4	4		2				18
15							2		2	2	5	1		1		13
17						1			1	1		1	2			6
19					1		1		1	1	2		2	1		9
21									1	2	1	1	1			6
23										1	1					2
25											1					1
27																
29											1					1
31																
33																
35															1	1
Frequency	1	2	6	21	63	34	51	15	37	22	29	6	9	2	2	300

Correlation = 0.550±0.027.

TABLE XV.—*Yield in decigrams of kernels per plant correlated with average weight in milligrams of kernels per plant. 1915*

Average weight of kernels per plant (mgm.).	Yield of kernels per plant (dgm.).																Frequency.
	8.75	11.25	13.75	16.25	18.75	21.25	23.75	26.25	28.75	31.25	33.75	36.25	38.75	41.25	43.75	46.25	
2.5	...	...	...	1	...	1	1	...	...	...	...	...	...	...	...	...	3
7.5	...	1	...	...	1	4	2	6	2	1	1	1	...	...	...	...	19
12.5	...	...	1	...	1	3	...	2	2	1	...	...	...	...	...	...	10
17.5	2	1	...	1	2	1	5	10	5	2	1	...	...	...	...	...	30
22.5	...	1	1	1	2	7	6	10	3	5	...	...	...	...	...	...	36
27.5	...	...	...	...	3	4	12	17	7	4	1	...	1	...	...	...	49
32.5	...	...	...	...	2	1	14	17	14	8	2	...	...	...	...	...	58
37.5	...	...	...	...	1	6	13	27	16	11	2	1	...	...	...	...	77
42.5	...	...	...	...	...	4	5	31	22	18	1	1	...	...	...	...	82
47.5	...	...	...	...	...	1	3	24	13	12	2	1	...	...	...	...	56
52.5	...	...	...	...	...	...	1	19	20	15	5	1	...	...	...	...	52
57.5	...	...	...	...	...	...	...	2	12	13	3	...	1	1	...	...	32
62.5	...	...	...	...	...	...	...	4	4	12	...	...	...	...	...	1	21
67.5	...	...	...	...	...	...	...	4	3	9	2	1	...	...	...	...	19
72.5	...	...	...	...	...	...	...	...	3	7	5	...	...	...	...	...	15
77.5	...	...	...	...	...	...	...	...	...	2	2	2	...	...	...	...	6
82.5	...	...	...	...	...	...	...	...	...	2	...	...	...	...	...	...	2
87.5	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	...	1
92.5	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	...	1
97.5	...	...	...	...	...	...	...	...	...	2	...	...	...	...	...	...	2
Frequency	2	3	2	3	12	32	62	164	126	125	28	8	2	1	...	1	571

Correlation = 0.504 ± 0.021.

TABLE XVI.—*Weight in decigrams of kernels per plant correlated with average weight in milligrams of kernels per plant. 1916*

Average weight of kernels per plant (mgm.).	Yield of kernels per plant (dgm.).																Frequency.
	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	
1.5	3	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	4
4.5	...	...	2	2	1	3	2	2	1	2	2	1	...	...	...	...	18
7.5	...	...	...	...	1	1	3	4	2	3	4	4	7	4	3	1	37
10.5	...	1	1	...	1	2	5	8	20	19	18	2	2	2	3	...	84
13.5	...	...	...	...	...	2	9	12	8	11	22	28	10	2	3	...	107
16.5	...	...	1	...	2	...	2	12	30	46	16	11	18	6	4	1	149
19.5	...	...	...	...	1	...	...	2	5	27	49	35	10	...	4	...	133
22.5	...	...	...	...	...	...	...	1	6	8	19	35	26	2	1	1	99
25.5	...	...	...	...	...	...	...	1	3	8	8	11	9	7	2	...	49
28.5	...	...	...	...	...	...	...	2	1	1	3	2	...	2	1	...	12
31.5	...	...	...	...	...	...	...	...	...	...	...	2	2	...	...	...	4
34.5	...	...	...	...	...	...	...	...	...	...	1	1	...	...	...	...	2
Frequency	[illegible]	1	4	3	6	8	21	44	76	125	142	132	84	25	21	3	698

Correlation = 0.370 ± 0.022.

TABLE XVII.—*Yield in decigrams of kernels per plant correlated with average weight in milligrams of kernels per plant. 1917*

Average weight of kernels per plant (mgm.)	Yield of kernels per plant (dgm.)																					Frequency
	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
1.5	1							1														2
4.5													1									1
7.5																	1					1
10.5										1		1	1			1	4	3				11
13.5										1	2	3	1	4	3	2	1	1				18
16.5							1					3	2	4	11	6	2					29
19.5						1		1				2	7	5	17	13	7	2				55
22.5										1	1	1	11	21	13	8	4	2				62
25.5			1						1	1	2	1	3	16	30	20	2	1				78
28.5										1		1	3	4	38	37	8	1				93
31.5													1	3	19	29	5				1	58
34.5													1	2	2	13	4	4	1			27
37.5													2	3	3	13	3	2	1			27
40.5													1	2	3	3	1	1				11
43.5														1	1			1				3
46.5															1							1
49.5																	1					1
52.5																						...
55.5																	1					1
Frequency	1	...	1	...	...	1	1	2	1	5	5	12	34	65	141	145	44	18	2	...	1	479

Correlation = 0.3064 ± .027.

TABLE XVIII.—*Average height in centimeters of culms per plant correlated with average weight in milligrams of kernels per plant. 1914*

Average weight of kernels per plant (mgm.)	Average height of culms per plant (cm)															Frequency
	5.25	6.75	8.25	9.75	11.25	12.75	14.25	15.75	17.25	18.75	20.25	21.75	23.25	24.75	26.25	
27.5					1											1
32.5																...
37.5																...
42.5				1	3											4
47.5		1		2	5	3	1									12
52.5			1	5	6	2	4		2	1						21
57.5		1	2	5	7	6	6		3	1	1		1			33
62.5	1		1	2	13	8	6	2	4	2	2					41
67.5			2		7	7	8	5	3	5	3	1	2			43
72.5				1	8	4	9	1	8	5	10	1	1			48
77.5				4	9	3	9	2	9	5	4	2	3			50
82.5				1	4		7	1	3	2	4		1		1	24
87.5						1		2	3	1	3	1	1	2	1	15
92.5							1	1	1		2					5
97.5								1								1
102.5												1				1
107.5									1							1
Frequency	1	2	6	21	63	34	51	15	37	22	29	6	9	2	2	300

Correlation = 0.458 ± 0.030.

TABLE XIX.—*Average height in centimeters of culms per plant correlated with average weight in milligrams of kernels per plant. 1915*

Average weight of kernels per plant (mgm.)	Average height of culms per plant (cm.) 8.75	11.25	13.75	16.25	18.75	21.25	23.75	26.25	28.75	31.25	33.75	36.25	38.75	41.25	43.75	46.25	Frequency
62.5						1			1		1						3
67.5								1	3								4
72.5		1			1		1	1	1			1					6
77.5	1					2	3	3	2	1	2						14
82.5						3	3	4	3	4	1						18
87.5	1		1		3	4	5	16	13	10	4	1					58
92.5				1		5	7	21	15	15	5	1					70
97.5				1	2	6	8	29	32	27	6	2		1		1	115
102.5		1	1		5	6	19	46	25	36	1	3	2				145
107.5				1	1	5	12	31	22	21	5						98
112.5		1					3	7	7	8	3						29
117.5								5	1	2							8
122.5							1			1							2
127.5									1								1
Frequency	2	3	2	3	12	32	62	164	126	125	28	8	2	1		1	571

Correlation=0.071±0.028.

TABLE XX.—*Average height in centimeters of culms per plant correlated with average weight in milligrams of kernels per plant. 1916*

Average weight of kernels per plant (mgm.)	Average height of culms per plant (cm) 14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	Frequency
50												1					1
54				1							1						2
58													1				1
62			1														1
66	1			1		3	1										6
70	1				1	1	1	1									5
74		1	1		1	1	7	6	3	7	1		1				29
78			1	1	1	3	8	21	21	20	11	3	2				92
82			1		1		2	9	42	44	39	12	1				151
86					1		1	6	8	43	61	61	19	4	1		205
90					1		1		2	10	26	39	42	10	8	1	140
94	1							1		1	3	13	15	10	9	1	54
98												3	3	1	2	1	10
102															1		1
Frequency	3	1	4	3	6	8	21	44	76	125	142	132	84	25	21	3	608

Correlation=0.648±0.014.

TABLE XXI.—*Average height in centimeters of culms per plant correlated with average weight in milligrams of kernels per plant. 1917*

Average weight of kernels per plant (mgm.)	Average height of culms per plant (cm) 20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	Frequency
37.5	1																					1
42.5																						
47.5								1														1
52.5																						
57.5																						
62.5																						
67.5																1						1
72.5																						
77.5												1	1									2
82.5							1			1	1	1	2	1	2		1					10
87.5								1		1	1	4	10	19	15	9	3	2				65
92.5			1						1	1	2	4	16	24	58	38	10	3			1	159
97.5						1				1	1	2	5	14	55	71	20	5	2			177
102.5										1				7	11	26	9	6				60
107.5																	1	2				3
Frequency	1		1			1	1	2	1	5	5	12	34	65	141	145	44	18	2		1	479

Correlation=0.426±0.025.

YIELD OF KERNELS CORRELATED WITH OTHER PLANT CHARACTERS

The coefficients for yield of kernels per plant correlated with number of kernels per plant are 0.851±.0.010 in 1914, 0.881±0.006 in 1915, 0.952±0.002 in 1916, and 0.973±0.001 in 1917. In contrast with the coefficients when weight of seed was correlated with plant characters, the correlation is consistently high in each of the four years. With a fair uniformity in the average weight of kernels, high correlation between yield of kernels and number of kernels is to be expected.

Yield of kernels correlated with average weight of kernels gave coefficients of 0.550±0.027 in 1914, 0.504±0.021 in 1915, 0.370±20.02 in 1916, and 0.306±0.027 in 1917. This order is the opposite of the general tendency for the coefficients in this group to be lower in the first two than in the last two years. The correlation between the two characters is substantial and fairly consistent. This indicates that the higher yielding plants have a tendency to produce kernels of greater average weight.

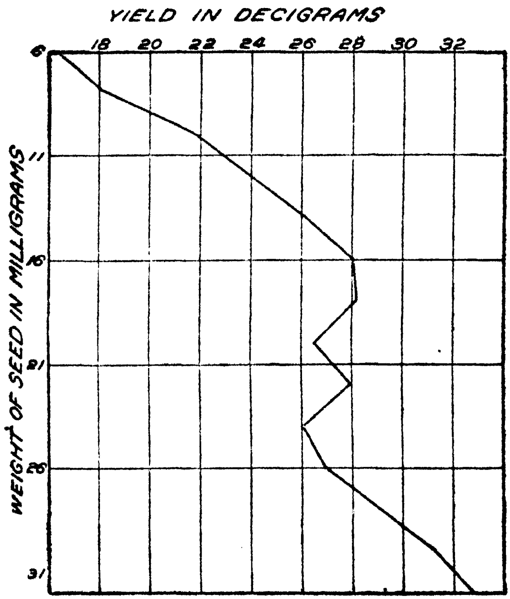

FIG. 8.—Graph showing regression for weight of seed and yield of kernels per wheat plant in 1917.

The coefficients for yield of kernels correlated with the number of culms are 0.500±0.029 in 1914, 0.669±0.015 in 1915, 0.818±0.008 in 1916, and 0.824±0.009 in 1917. The correlation between the two characters is relatively high, but not as consistent as that between yield of kernels and number of kernels. Plants with the greater number of culms are usually the higher yielders.

For yield of kernels correlated with average height of culms the coefficients are 0.303±0.025 in 1915, 0.384±0.033 in 1914, 0.452±0.024 in 1917, and 0.478±0.019 in 1916. This is a substantial and fairly consistent correlation. For yield of kernels and average length of spikes, the coefficients are very similar to those between yield of kernels and average height of culms. There is a distinct tendency for the plants

producing the higher yields of grain to have the greater average height of culms and greater average length of spikes. Stated in another way, the plants having the greater average height of culms and average length of spikes have a tendency toward being the highest yielders.

The coefficients for yield of kernels correlated with total length of spikes per plant are 0.636±0.023 in 1914, 0.808±0.009 in 1915, 0.910±0.004 in 1916, and 0.911±0.005 in 1917. Correlation between these two characters is high and relatively consistent approaching that between yield of kernels and number of kernels. Stated directly, the plants with the greatest total length of spikes were generally the highest yielders.

The results for yield of kernels correlated with the several characters may be summarized as follows: An increased yield of kernels is very closely accompanied by an increase in number of kernels, number of culms, and total length of spikes, and somewhat less closely accompanied by an increase in average weight of kernels per plant, average height of culms, and average length of spikes.

NUMBER OF CULMS CORRELATED WITH OTHER PLANT CHARACTERS

For number of culms correlated with average length of spikes per plant, the coefficients are 0.061±0.038 in 1914, 0.024±0.028 in 1915, 0.039±0.025 in 1916, and 0.236±0.029 in 1917. In the first three years there is practically none, and in the last year a low correlation. The conclusion is that these two characters move practically independent of each other.

The coefficients for number of culms correlated with total length of spikes per plant are 0.872±0.009, 0.839±0.008, 0.958±0.002, and 0.946±0.003, respectively, for the 4-year period. The correlation between the two characters is somewhat more close and consistent than that between yield of kernels and total length of spikes. An increase in number of culms is followed by an increase in total length of spikes per plant, but not by greater average length of spikes.

AVERAGE WEIGHT OF KERNELS CORRELATED WITH OTHER PLANT CHARACTERS

The coefficients for average weight of kernels as correlated with number of kernels per plant are 0.137±0.038, 0.192±0.027, 0.160±0.024, and 0.160±0.030, respectively, for the four years. The coefficients are uniformly low but positive in each instance with the lowest 3.6 times its probable error. To a limited extent, an increase in number of kernels is accompanied by a greater average weight of the kernels.

Average weight of kernels correlated with number of culms per plant gave coefficients of −0.071±0.038 in 1914, 0.137±0.027 in 1915, −0.054±0.025 in 1916, and −0.009±0.030 in 1917. The low coefficients as judged by their probable errors and the variation from year shows slight or no correlation between these characters.

The coefficients for average weight of kernels correlated with average length of spikes are 0.153±0.038, 0.120±0.027, 0.552±0.017, and 0.411±0.025, respectively, for the four years. The relatively low correlation in the first two and the substantial correlation in the last two years indicates that under the conditions of environment which prevailed in 1916 and 1917 there is a strong tendency for the two characters to move together; and that under extreme environmental conditions such as prevailed in 1914 and 1915 the relation is considerably reduced.

For average weight of kernels correlated with total length of spikes, the coefficients range from 0.001±0.038 in 1914 to 0.159±0.027 in 1915. There is no correlation in 1914, and for the three other years the relation is low. Therefore the conclusion must be that the two characters move practically independent of each other.

When average weight of kernels is correlated with number of kernels, number of culms, average length of spikes, and total length of spikes, no consistently high relationship is found. Subject to radical change by environment, there is a moderate relation with average length of spikes. With number of kernels the correlation is rather low but consistent. Average weight of kernels is practically independent of total length of spikes.

AVERAGE HEIGHT OF CULMS CORRELATED WITH OTHER PLANT CHARACTERS

The coefficients for average length of culms correlated with number of kernels per plant are 0.257±0.036, 0.339±0.025, 0.364±0.022, and 0.431±0.025, respectively, for the four years. This is a substantial and fairly consistent relation very similar to that found between yield of kernels and average height of culms. There is a tendency for an increase or a decrease in average height of culms to result in the production of a larger or smaller number of kernels per plant.

When average height of culms is correlated with average weight of kernels, the coefficients are 0.458±0.030 in 1914, 0.071±0.028 in 1915, 0.648±0.014 in 1916, and 0.426±0.025 in 1917. With the exception of the very low correlation in 1915 due to extremely favorable environmental conditions, the relation is substantial. The indications are that under ordinary conditions there is a tendency for increase or decrease in average height of culms to be accompanied by a raising or lowering of average kernel weight.

For average height of culms correlated with number of culms per plant the coefficients are −0.195±0.037 in 1914, −0.092±0.028 in 1915, 0.046±0.025 in 1916, and 0.205±0.029 in 1917. The correlation varies considerably from year to year and is low in each instance. Therefore the conclusion may be drawn that the slight tendency for the two characters to vary together is highly modified by the influences of environment.

The coefficients for average height of culms correlated with average length of spikes per plant are 0.315 ± 0.035 in 1914, 0.419 ± 0.023 in 1915, 0.775 ± 0.010 in 1916, and 0.668 ± 0.017 in 1917.

Similar to the correlation between yield of kernels and average length of spikes, the relation of these two characters has a tendency to be high, but is strongly modified by environmental conditions.

When average height of culms is correlated with total length of spikes per plant, the coefficients are 0.036 ± 0.038, 0.260 ± 0.026, 0.235 ± 0.024, and 0.351 ± 0.027. This is a variation from no correlation to a fairly substantial one. The influence of environment may entirely overcome the tendency of the two characters to move together.

Considering as a whole the relation of average height of culms to other plant characters, there is a tendency for an increase or decrease in average height of culms to be accompanied by an increase or decrease in number of kernels and average length of spikes. Between average height of culms and average weight of kernels there is a substantial correlation and between average height of culms and total length of spikes there is a moderate correlation three years out of four. The correlation between average height of culms and number of culms is always low.

CORRELATION OF HEIGHT OF PLANTS AT DIFFERENT STAGES OF DEVELOPMENT

When height at appearance of second leaf is correlated with height of the same plants at six weeks from seeding, the coefficients are 0.406 ± 0.038 in 1914, 0.467 ± 0.022 in 1915, 0.470 ± 0.019 in 1916, and 0.466 ± 0.024 in 1917. The correlation between the two characters is substantial and consistent.

For height of plants at appearance of second leaf correlated with height of tallest culm at maturity, the coefficients are 0.380 ± 0.033 in 1914, 0.270 ± 0.026 in 1915, 0.272 ± 0.023 in 1916, and 0.211 ± 0.029 in 1917. This is a medium correlation modified considerably by environmental influences.

The coefficients for height at six weeks correlated with height of the tallest culms of the same plants at maturity are 0.399 ± 0.038, 0.236 ± 0.026, 0.523 ± 0.018, and 0.314 ± 0.027. The correlation between the two characters varies from rather low to moderately high depending upon the environment.

Considering as a whole the correlations between height of plants at different stages of development, there is a distinct tendency for plants of varying heights at second leaf to maintain the same relative heights at six weeks, but there is a lesser tendency for this relation to be maintained at maturity. Some of the shorter plants at second leaf approach closely or equal in height the taller ones at maturity. There is a tendency, considerably modified by environment, for differences in heignt of plants at six weeks to be maintained in the tallest culm at maturity.

Considering the interrelation of plant characters as a whole, there is a range from practically none to a high correlation. Correlation is modified by environment, the degree of modification due to this cause varying with the characters considered.

An increased yield of kernels is very closely accompanied by an increase in number of kernels, number of culms, and total length of spikes; and somewhat less closely accompanied by increase in average weight of kernels per plant, average height of culms, and average length of spikes.

A larger number of culms per plant is accompanied by a greater total length of spikes but not by a greater average length of spikes.

Average weight of kernels is substantially and fairly consistently correlated with yield of kernels; and, subject to radical change due to environment, moderately correlated with average length of spikes. With number of kernels, the correlation is rather low but always consistent. Average weight of kernels is practically independent of average length of spikes.

There is a distinct tendency for greater average height of culms to be accompanied by a greater average length of spikes, number of kernels, and a higher yield of kernels. Average height of culms is substantially correlated with average weight of kernels and moderately correlated with total length of spikes in three years out of four. The correlation between average height of culms and number of culms is always low.

There is a distinct tendency for plants of varying heights at second leaf to maintain the same relative heights at six weeks; but there is a lesser tendency for this relation to be maintained at maturity.

GENERAL DISCUSSION

During early growth in 1914 and 1915, the means for height of the plants are greater than those in 1916 and 1917, owing to the somewhat more productive soil on which the plants were grown, to the more favorable weather conditions, and to a higher average weight of seed planted. In 1915 the favorable growing conditions continued throughout the season, and the mean for each plant character at maturity, except average weight of seed, is the highest in the 4-year period. In 1914, during July, drouth followed by an epidemic of black-stemrust lowered materially the means for all plant characters at maturity.

For each of the characters studied, except yield of kernels, the variability as indicated by the standard deviations, is as high as or higher in 1914 and 1915 than in 1916 and 1917. The generally higher variability in the former two as compared with that in the latter two years is accompanied by generally lower correlation coefficients (1) when weight of seed sown is correlated with resultant plant characters and (2) when yield of kernels is correlated with other plant characters.

When weight of seed sown is correlated with plant characters at maturity, it is noticeable that in 1915 there are four coefficients with the minus sign and that there is a tendency for the coefficients to be lower than in 1914.

In contrast with the low and varying relation in 1914 and 1915 is the generally moderate and consistent correlation between weight of seed sown and plant characters in 1916 and 1917 when the plants were grown on the poorer soil and from somewhat lower mean weight of seed.

From this study conclusive evidence is given that for the conditions under which the work was done, environment reduced radically or obliterated entirely the correlation between weight of seed sown and plant characters among which is yield.

This information answers, in part at least, the questions raised in the introduction to this article regarding the rôle of weather and soil in comparisons of heavy and light seed for planting.

If these results were applicable to the wheat crop in general during the 4-year period, it is clear that on soils of moderately high productivity with favorable weather conditions heavy kernels as compared with light kernels used for planting may be expected to give very moderate or no increase in yield.

In the study of the interrelation of plant characters a substantial and fairly consistent correlation was found between yield of kernels and average weight of kernels, average height of culms, and a somewhat higher correlation with number of culms. Between average height of culms and average weight of kernels there is a moderately high correlation each year, except in 1915, when the coefficient is very low.

If these relations held for the wheat crop during the 4-year period, separating from the crop each year seed of higher average weight would be selecting seed from plants which had a decided tendency toward higher yield, and, with the exception of the year 1915, from plants which were taller and at the same time higher yielding. In 1915 there was practically no relation between average weight of kernels and average height of culms, and separating the larger seeds from this crop would be selecting seed from both high and low yielding plants.

The tendency of the tallest plants and the plants having the greatest number of culms to be the highest yielders is a valuable index in making individual plant selections from mixed populations.

SUMMARY OF CONCLUSIONS

Subject to the environmental conditions under which the work was done, the following conclusions may be drawn:

(1) The magnitude of the means for any of the characters studied varied in response to environmental conditions. Lower yields of straw resulted from a reduction in number, total length, or average length of culms per plant; and lower yields of grain from a reduction in the

number of kernels. When the kernels developed normally, lower yield was accompanied by a higher average weight per kernel.

(2) In general, a reduction in the magnitude of the means is accompanied by less variability. A number of exceptions to this general tendency occurred.

(3) Correlation between weight of seed sown and resultant plant characters at maturity is not high in any instance and may be so modified by environmental conditions that the relation may be slight or obliterated entirely.

(4) Correlation between plant characters is modified by environment, the degree of modification from this cause varying with the characters considered.

(5) An increased yield of kernels is very closely accompanied by an increase in number of kernels, number of culms, and total length of spikes; and somewhat less closely accompanied by an increase in average weight of kernels per plant, average height of culms, and average length of spikes.

(6) A larger number of culms per plant is accompanied by a greater total length of spikes but not by a greater average length of spikes.

(7) Average weight of kernels is substantially and fairly consistently correlated with yield of kernels, and, subject to radical change due to environment, moderately correlated with average length of spikes. With number of kernels the correlation is rather low but always consistent. Average weight of kernels is practically independent of average length of spikes.

(8) There is a distinct tendency for greater average height of culms to be accompanied by greater average length of spikes, number of kernels, and higher yield of kernels. Average length of spikes is moderately correlated with average weight of kernels three years out of four. The correlation between average height of culms and number of culms is always low.

(9) There is a distinct tendency for plants of varying height at second leaf to maintain the same relative heights at six weeks, but there is a lesser tendency for this relation to be maintained at maturity.

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OBTAINING BEET LEAFHOPPERS NONVIRULENT AS TO CURLY-TOP

[PRELIMINARY PAPER]

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The beet leafhopper (*Eutettix tenella* Baker) is the only known agent capable of transmitting the disease of the sugar beet (*Beta vulgaris*) known as curly-top. The fact that under some conditions, when collected from wild vegetation, the insects of this species failed to occasion the beet disease until they had fed on diseased plants was first shown by Boncquet and Hartung [1] and later confirmed by Smith and Boncquet.[2] Experiments which had been started previously by Stahl to determine whether or not a leafhopper which has never fed on beets affected with curly-top will produce the disease proved the point, which was inferred from the discovery mentioned above, that it will not. Further tests made by the present writers to verify the earlier results have led to the development of the method, here to be described, of obtaining nonvirulent leafhoppers with certainty and relative ease.

The manner in which the egg of the leafhopper hatches makes it possible to remove the young nymph from the diseased to a healthy plant before it has had an opportunity to feed. The eggs are laid mainly in the petioles and midribs of the leaves. In the process of hatching the nymph forces its way, anterior end first, from the egg case and through the slit of the ovipositor. This is accomplished by an undulating movement of the body. Emergence from the egg membrane is practically complete, and the body of the insect reaches a position more or less perpendicular to the plant surface before the appendages begin to unfold. As the appendages unfold the contortions of the body become more vigorous until the young nymph gains a foothold on the substratum. It is then able to free itself entirely from the egg membrane. The process is completed after from 5 to 16 minutes. During the latter part of the operation, when the appendages are unfolding, the opportunity is afforded of lifting the nymph off and transferring it to a healthy plant. Its transfer can be best effected by means of a small camel's-hair brush.

The first experiment, by Stahl, was begun on April 19, 1915. On that day three lots of nymphs, numbering 7, 9, and 15 individuals, respec-

[1] BONCQUET, P. A., and HARTUNG, WM. J., THE COMPARATIVE EFFECT UPON SUGAR BEETS OF EUTETTIX TENELLA BAKER FROM WILD PLANTS AND FROM CURLY-TOP BEETS. *In* Phytopathology, v. 5, no. 6, p. 348–349. 1916.

[2] SMITH, RALPH E., and BONCQUET, P. A. CONNECTION OF A BACTERIAL ORGANISM WITH CURLY LEAF OF SUGAR BEET. *In* Phytopathology, v. 5, no. 6, p. 335–341. 1915.

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tively, were transferred as they hatched to three healthy beet plants in separate cages. The insects were left on the plants until after they had become adults. All three plants remained healthy. On July 3 the insects of two of the lots were caged on two separate plants affected with curly-top. After 17 days they were again caged on two healthy plants. Both of these plants developed the disease.

A similar test was begun on May 17, 1915. Three lots of approximately 15 nymphs each were placed on healthy beets as before. At the same time two similar lots were transferred to diseased beets as controls. After the insects of the latter two lots were about half grown they were shifted to two healthy plants. These plants became diseased, while the three plants on which the first three lots were placed and kept remained healthy.

Recently some work was performed jointly by the writers to verify the earlier results and to secure a supply of nonvirulent leafhoppers for laboratory experiments. Three lots of nymphs, numbering approximately 50, 100, and 200, respectively, were lifted off in the manner described and placed on three healthy beet plants. The three lots were kept separate, and about 60 per cent of each grew to maturity on the original plants or on fresh healthy plants which were substituted as needed. In no case has a plant on which these insects were caged developed curly-top. During the same time the disease developed in other plants with which virulent insects had been caged under similar conditions. Single leafhoppers from each of the three lots have been caged on healthy plants without apparent effect, while at the same time virulent insects, caged individually on healthy plants, have quickly induced the disease. After having been caged on diseased plants, however, the nonvirulent insects have become virulent.

These results show conclusively that uninfected insects placed on healthy beet plants will not produce curly-top. They are of further interest because the possibility of obtaining a supply of leafhoppers known positively to be nonvirulent opens up several promising lines of attacking the disease problem. For instance, nonvirulent leafhoppers may be used to determine whether or not other plants than beets harbor the virus of curly-top. The peculiar disease of the common mallow (*Malva parviflora* L.) was thus shown by Boncquet and Stahl [3] to be caused by the same virus which causes the beet disease.

[3] BONCQUET, P. A., and STAHL, C. F. WILD VEGETATION AS A SOURCE OF CURLY-TOP INFECTION OF SUGAR BEETS. *In* Jour. Econ. Ent., v. 10, no. 4, p. 392–397, pl. 17–18. 1917.

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ACIDITY OF SILAGE MADE FROM VARIOUS CROPS[1]

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INTRODUCTION

It has been found that the quality of corn silage is chiefly dependent upon the kind of acids formed during the fermentation (*3, 4, 10*).[2] The purpose of this investigation is to ascertain whether the same acids are developed when other commonly grown crops are used for silage purposes. Corn (*Zea mays*) is the leading crop grown for silage, owing to the heavy yield of green material obtained per acre, but in many sections of the Pacific Northwest the growing of corn is prohibitive because of variable climatic conditions caused by different altitudes. In these sections, when silage is desired, crops other than corn must necessarily be grown.

REVIEW OF LITERATURE

There are comparatively few references in the literature upon the development of acidity in silage made from crops other than corn.

Esten and Mason (*7*) have recommended mixing a legume with corn for the purpose of raising the protein content of silage. They asserted that three parts of corn with two parts of cowpeas or soybeans made an excellent combination; also that rye or wheat when mixed with clover made a good silage mixture. They reported that the most progressive farmers of the State were successfully siloing a legume with some member of the grass family. Reports from many States and correspondence with the Experiment Stations indicate that there is a growing tendency to silo cowpeas which have been grown with corn. No data were given to show the type of acid fermentation in any of the above-mentioned crop mixtures.

Recently the Kansas Agricultural Experiment Station (*11, 12*) found that good silage resulted when corn or molasses was mixed with alfalfa in the proportion of 1 to 10 or 1 to 20. The individual acids, however, were not determined, the total acidity being calculated as lactic acid.

[1] Published with the approval of the Director of the Idaho Experiment Station.

[2] Reference is made by number (italic) to "Literature cited," p. 23.

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Upson (*14*), of the Nebraska Agricultural Experiment Station. carried on an investigation with alfalfa and Black Amber sorghum cane mixtures and with alfalfa alone. His results showed excellent silage when alfalfa was mixed with different amounts of Black Amber sorghum cane, but when alfalfa was siloed alone, an undesirable product resulted. His results on the acidity were expressed as total acidity calculated in terms of acetic acid.

True, Woll, and Dolcini (*13*), of the California Experiment Station, have reported favorably upon the practice of cutting the first crop of alfalfa for silage, for the reason that the crop is generally weedy and makes a very inferior hay. Their experience showed that silage made from the first cutting of alfalfa which consisted in a large part of foxtail and other weeds made good silage. The chemical analyses showed that both volatile and nonvolatile acids were present.

A review of the literature indicates that alfalfa can be made into good silage if some material having a high percentage of fermentable carbohydrates is mixed with it.

The siloing of clover has received more favorable comment. Clark (*2*), of the Montana Experiment Station, has summarized the data on clover silage. He reports that at the Agassiz Experiment Station, British Columbia, clover is commonly used for silage. He also states that good results were obtained with it at Pennsylvania State College. The only objection to its use was that a strong odor developed. However, cows ate it readily. Reports from Wisconsin show that tests were made years ago with the uncut plant with unsatisfactory results. Recently more favorable results were obtained when clover was cut in 1-inch lengths and was well tramped in the silo.

EXPERIMENTAL WORK

The work of the Idaho Station on the determination of acids in silage was planned in 1915. At that time only corn silage was available for analysis. The corn was siloed in the fall of 1915 in a large concrete silo of the monolithic type located upon the University farm. The silage had been partially fed to stock before the analysis was begun.

METHOD OF OBTAINING SAMPLES FOR THE ACID DETERMINATION

A composite sample was collected from the various parts of the surface of the silo. One hundred gm. were weighed out and dried to constant weight at 100° C. for the moisture content. The remaining portion of the sample was placed in a hydraulic press and the juice pressed out. To 100 gm. of the juice a small quantity of normal sulphuric acid was added, and the volatile acids separated from the nonvolatile acid by distillation in a current of steam under reduced pressure.

Four liters of distillate were collected and neutralized with *N/10* barium hydroxid and evaporated to a small volume. The volatile acids

were then freed from their barium salts by the addition of a theoretical amount of sulphuric acid. After filtering off the barium-sulphate precipitate, the solution was made up to a definite volume and the volatile acid determined quantitatively. The juice which remained after separating the volatile acids from the nonvolatile acid under reduced pressure was used for the nonvolatile-acid determination.

METHODS USED IN DETERMINING THE ACIDS OF SILAGE

VOLATILE ACIDS

The Duclaux method was used in determining the volatile acids. Until the recently proposed method of Dyer (*5*) it was the only method which proved applicable in the estimation of small amounts of volatile acids when present in a mixture. This method was previously used in studies on corn silage, and since a complete discussion has been taken up under the previous citations, no explanation of the principles involved will be given here. It has been used by numerous investigators in determining volatile acids in known and unknown mixtures. Criticisms have been made of the Duclaux method by Upson, Plum, and Schott (*15*). Some of the criticisms concerning the difficulties involved in carrying the analysis by the Duclaux method the writer fully appreciated in previous work with mixtures of volatile acids. But experiments in determining known mixtures of acids have shown that under the most carefully regulated conditions of distillation, accurate results are obtained and the writer believes that the method deserves greater confidence than given it by the above-mentioned investigators.

Voitkevich (*16*) has obtained results on known mixtures of acetic, propionic, and butyric acids which differed not more than 5 per cent from each other, and this difference is attributed to the variations in the conditions of distillations. He concludes that the method will yield accurate and trustworthy results when carried on under carefully regulated conditions.

Dyer has proposed an excellent method which is more simple to manipulate. The method involves the distillation of the acids in a current of stream from a constant volume. The titration figures are plotted in the form of curves which are characteristic for each acid.

More recently Gillespie and Walters (*9*) published methods for calculating algebraically and graphically the amount of volatile acids in a mixture. The methods of calculation suggested by them are applicable to either the Duclaux or Dyer method, as the methods of calculation are applied irrespective of the mode of distillation. It is merely necessary to conduct all distillations of pure acids and mixtures in the same manner.

COMPARISON OF RESULTS BY THE DUCLAUX AND THE DYER METHOD

A comparison of the Duclaux and the Dyer method was made on a solution of volatile acids obtained from silage in the following manner. Three hundred gm. of expressed juice from silage were acidified slightly with normal sulphuric acid and distilled in a current of steam under reduced pressure. Four liters of the distillate were collected. Five hundred cc. of this distillate were carefully neutralized with $N/10$ sodium hydroxid, and evaporated to a small volume. The volatile acids were liberated from their sodium salts by adding the theoretical amount of sulphuric acid. The quantitative determination of volatile acids was then made by Dyer's method.

One thousand cc. of the 4-liter distillate were carefully neutralized with barium hydroxid, evaporated to a small volume, and the acids freed from the barium salts by the addition of the required quantity of normal sulphuric acid. After filtering off the barium sulphate, the solution was made up to volume and the acids determined by the Duclaux method.

A comparison of the results by the two methods is given below.

Volatile acids in 100 gm. of pea-silage juice, as determined by—

	Dyer method.	Duclaux method.
	Gm.	*Gm.*
Acetic acid	0.629	0.601
Propionic acid	.033	.037
Total volatile acids	.662	.638

Volatile acids in corn-silage juice, as determined by—

	Dyer method.	Duclaux method.
	Gm.	*Gm.*
Acetic acid	0.824	0.796
Propionic acid	.072	.082
Total volatile acids	.896	.878

The above results are typical of several determinations of the quantities of volatile acids in the juices of different kinds of silage by the two methods. In all determinations the orientation tests as described by Dyer were made for the individual volatile acids, and their presence or absence was confirmed. The results indicate a slight difference in the proportions of acids found by the two methods; yet this difference easily falls within the limits of experimental error, and it is obvious that either method is applicable for a comparative study of volatile acids in silage.

NONVOLATILE ACID

Lactic acid was determined in the juice that remained after distilling off the volatile acids under reduced pressure. This solution was evaporated on a water bath to a small volume, then extracted with ether in a Bremer continuous extractor for 72 hours. After distilling off the ether, the acid solution was diluted with water, boiled with an excess of barium hydroxid, then exactly neutralized with sulphuric acid. The barium sulphate was filtered off and zinc sulphate added to the filtrate, care being taken to avoid an excess. After filtering off the barium sulphate, the solution was evaporated slowly, the zinc lactate being allowed to crystallize. The crystals were filtered off and washed with a small portion of cold water, then dried, and weighed. A second and sometimes a third crop was obtained from the mother liquor.

OPTICAL ACTIVITY OF LACTIC ACID FROM SILAGE

The combined crops of zinc lactate from each determination were examined for their optical activity. A solution containing at least 4 per cent of zinc lactate in a 2-dm. tube invariably gave a reading of zero degrees. Two gm. of zinc-lactate crystals were also dried in an oven to determine the water of crystallization. Two gm. of zinc lactate gave 0.3635 gm. of water, or 18.17 per cent.

Theoretical water of crystallization for zinc-lactate crystals

	Active form.	Inactive form.
Water of crystallization	12.89	18.18

Although the 2 gm. of zinc lactate were taken from a combined portion of samples obtained from each silo, it is safe to conclude that the zinc lactate was the inactive form. Such results are to be expected in silage fermentation where the conditions of inoculation are not controlled.

EXAMINATION OF SILAGE FROM LARGE SILOS

INVESTIGATIONS OF 1915

CORN SILAGE

Although corn silage has been studied and reported by the Iowa Agricultural Experiment Station (*3*, *4*, *10*), it was thought best to include corn silage in this investigation. The maturity of the corn is not always the same in this country, owing to early frosts, and since maturity of corn has been stated by investigators to influence the amount of acidity formed, results on acidity of corn produced in Idaho were desired for the sake of comparison. The corn silage was made from a crop of selected Disco Pride corn, cut when the kernels were in the glazed stage. The results are given in Table I.

TABLE I.—*Acidity produced by corn silage in Idaho, 1915*

CONDITIONS OF EXPERIMENT

	Sample 1.	Sample 2.	Sample 3.	Sample 4.	Sample 5.
Date of sampling	Jan. 26, 1915.	Feb. 11, 1916.	Feb. 17, 1916.	Mar. 2, 1916.	Mar. 7, 1916.
Distance from bottom of silo..feet..	13	10	9	7	6
Moisture..per cent..	77.7	80.0	77.0	77.8	81.0
Dry material..do..	23.3	20.0	23.0	22.2	19.0

ACIDS IN 100 GM. OF SILAGE JUICE

	Gm.	Gm.	Gm.	Gm.	Gm.
Acetic acid	0.525	0.766	1.034	1.100	0.946
Propionic acid	.055	.058	.064	.076	.068
Butyric acid	.000	.000	.000	.000	.000
Total volatile acid	.580	.824	1.098	1.176	1.014
Lactic acid	1.141	1.535	1.329	1.595	1.341
Total acidity	1.721	2.359	2.427	2.771	2.355

ACIDS IN 100 GM. OF SILAGE CONTAINING MOISTURE

	Gm.	Gm.	Gm.	Gm.	Gm.
Acetic acid	0.408	0.613	0.796	0.856	0.766
Propionic acid	.043	.046	.049	.059	.055
Butyric acid	.000	.000	.000	.000	.000
Total volatile acid	.451	.659	.845	.915	.821
Lactic acid	.885	1.228	1.024	1.241	1.086
Total acidity	1.336	1.887	1.869	2.156	1.907

ACIDS IN 100 GM. OF SILAGE, DRY BASIS

	Gm.	Gm.	Gm.	Gm.	Gm.
Acetic acid	1.82	3.064	3.462	3.853	4.03
Propionic acid	.19	.232	.214	.266	.290
Butyric acid	.000	.000	.000	.000	.000
Total volatile acid	2.01	3.296	3.676	4.119	4.32
Lactic acid	3.97	6.140	4.460	5.580	5.72
Total acidity	5.98	9.436	8.136	9.699	10.14

INVESTIGATIONS OF 1916

In the work of 1916 the crops used for silage were corn, oats (*Avena sativa*), and peas (*Pisum* spp.), and wheat (*Triticum aestivum*) and peas. These crops were siloed in the three large concrete silos located on the University farm. Particular attention was given to the factors concerned in developing good silage—namely, fineness of cutting, thoroughness of packing, and the proper moisture content.

CORN SILAGE

Corn used for silage in 1916 was the Disco Pride corn, cut when the kernels were in the glazed stage. The amount and kinds of acid are given in Table II.

OATS AND PEAS

The oat and pea silage was made from a crop of white Canada field peas and Swedish Select oats sown at the rate of 60 pounds of the former and 40 pounds of the latter per acre. The crop was cut when the peas were beginning to harden in the pods and when the oats were in the dough stage. The results on acidity are given in Table II.

WHEAT AND PEAS

The wheat and pea mixture was made from a crop of white Canada field peas and Palouse Bluestem wheat sown at the rate of 75 pounds of the former and 25 pounds of the latter per acre. The peas grew luxuriantly, and the green weight of the peas exceeded considerably the green weight of the wheat. The crop was cut when the peas were beginning to harden in the pods and when the wheat was in the dough stage. The data on acidity are given in Table II.

TABLE II.—*Acidity produced by corn silage, oat and pea silage, and wheat and pea silage in large silos, Idaho, 1916*

Acid.	CORN SILAGE. (Date of sampling, Dec. 4, 1916; moisture, 77 per cent; dry material, 23 per cent; location, 15 feet from top of silo).			OAT AND PEA SILAGE. (Date of sampling, Oct. 6, 1916; moisture, 76 per cent; dry material, 24 per cent).			WHEAT AND PEA SILAGE. (Date of sampling, Nov. 11, 1916; moisture, 73.3 per cent; dry material, 26.7 per cent; location, 6 feet from bottom of silo).		
	Acidity in 100 gm. of—			Acidity in 100 gm. of—			Acidity in 100 gm. of—		
	Silage juice.	Silage containing original moisture.	Silage on dry basis.	Silage juice	Silage containing original moisture.	Silage on dry basis.	Silage juice.	Silage containing original moisture.	Silage on dry basis.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Acetic	1.022	0.787	3.46	0.542	0.396	1.47	0.718	0.526	1.97
Propionic	.053	.041	.18	.038	.028	.10	.052	.038	.14
Total volatile	1.075	.828	3.64	.580	.424	1.57	.770	.564	2.11
Lactic	1.280	.986	4.33	1.690	1.234	4.57	1.420	1.048	3.90
Total acidity	2.355	1.814	7.97	2.270	1.660	6.14	2.190	1.612	6.01

DISCUSSION OF RESULTS

CORN SILAGE.—All samples of corn silage examined in 1915 and 1916, showed the usual acid fermentation. There was more lactic acid formed than acetic and propionic acids, and butyric acid was absent from all

samples. Considerable more acidity was developed in this corn silage made in Idaho than in that studied previously at the Iowa Station. But this is partially accounted for by the fact that corn does not usually reach as high a state of maturity in this section. These results corroborate previous statements by certain investigators that immature corn will produce a silage higher in acidity than mature corn.

OAT AND PEA SILAGE.—Oat and peas made first-class silage. It had a good color and odor and showed an acid fermentation similar to corn silage.

WHEAT AND PEA SILAGE.—Wheat and peas made very good silage showing all the characteristics of a normal silage. The kinds and quantity of acids developed were similar to those found in corn silage.

EXAMINATION OF SILAGE FROM SMALL SILOS

In 1916 additional work was done with crop mixtures other than corn siloed in small wooden stave silos of approximately 1,500 pounds' capacity. In these silos peas and oats were siloed alone and in definite proportions, based on the dry weight of each, as were also clover and alfalfa, and definite mixtures of each, with wheat straw. Oats and peas were chosen because they were likely to be more commonly used than any other crops or crop mixture as corn substitutes. Clover and alfalfa were chosen because there are some sections where the first cuttings are cured with difficulty for hay because of rainy weather, and there has been some inquiry as to the possibility of converting them alone and in mixtures into silage. It seemed worth while to ascertain whether the same type of fermentation could be depended upon from mixtures of these legumes with crop residues that are all too frequently allowed to go completely to waste. The small silo series was filled with crops and mixtures as indicated below:

Peas 100 per cent.
Oats 100 per cent.
Clover 100 per cent.
Alfalfa 100 per cent.
Peas 87½ and oats 12½ per cent.
Peas 75 and oats 25 per cent.
Peas 50 and oats 50 per cent.
Clover 75 and wheat straw 25 per cent.
Clover 50 and wheat straw 50 per cent.
Alfalfa 75 and wheat straw 25 per cent.
Alfalfa 50 and wheat straw 50 per cent.

These minature silos were made of 2-inch fir staves. They were 3 feet in diameter and 6 feet in height. The staves were drawn closely together by means of lugs attached to iron bands. To make them perfectly air- and water-tight, the joints were coated on the inside with hot paraffin.

Before filling the silos, moisture determinations were made on the green materials in order to weigh out the proper amount. The cut materials were mixed uniformly on the floor and then packed in small

silos. One man was kept busy tramping and water was added when necessary in sufficient quantities to raise the average moisture content to 75 per cent. It was not necessary to add water to the peas, clover, and alfalfa when siloed alone.

When the silo was filled, a tightly fitting lid made of 2-inch fir plank was placed on the silage and 800 pounds of brick were evenly distributed on the lid. This pressure insured the proper settling of the silage, and made the conditions very similar to those found in a big silo, and in addition reduced the spoiled silage to a minimum.

Babcock and Russel (*1*) assert that silage made in small containers will be equal to the silage made in large silos, if conditions of siloing are properly controlled. Eckles and his collaborators (*6*) in their investigation on corn silage used small wooden silos 3 feet in diameter and 6 feet in height with the addition of a weight on the top of the silo to bring the silage under similar conditions as are found in the large silos. Their conclusions were as follows:

> A comparison of silage from a large silo and of silage from the same corn put into a small experimental silo showed the quality to be the same, as judged by appearance and by chemical analysis. For all purposes, except studying temperature changes, the small silo is believed sufficiently accurate for experimental purposes.

State of Maturity of the Different Crops

Peas were cut at the time the peas were beginning to harden in the pod, and the foliage around the bottom just beginning to turn brown.

Oats were cut when the kernels were in the dough stage.

Clover was the first cutting, cut at the stage when a few of the blossoms were beginning to turn brown.

Alfalfa was also from the first cutting, cut at the time when the new shoots began to appear.

Wheat straw used in the clover and alfalfa series contained a small quantity of wheat.

Examination of Silage

All silos were allowed to remain closed for a period of three months, with the exception of the clover silo, which was closed for four months. In all cases sufficient time elapsed to insure a complete acid fermentation. Samples were obtained by boring into the silos at a height of 3 feet from the floor, and by means of the auger removing quantities sufficient for the analyses. The holes in the sides of the silos were then stoppered with wooden plugs.

The silage samples were treated in the same manner as described in the early part of this paper for the determination of volatile and nonvolatile acids.

TABLE III.—*Acidity produced by pea silage, oat silage, oat and pea silage, clover silage, clover and wheat-straw silage, alfalfa silage, alfalfa and wheat-straw silage in small silos, Idaho, 1916*

Acid.	PEA SILAGE. (Moisture, 76 per cent; dry material, 24 per cent.) Acidity in 100 gm. of—			OAT SILAGE. (Moisture, 70.4 per cent; dry material, 29.6 per cent.) Acidity in 100 gm. of—			OAT AND PEA SILAGE (PEAS, 87½ PER CENT; OATS, 12½ PER CENT, DRY BASIS). (Moisture, 76.2 per cent; dry material, 23.8 per cent.) Acidity in 100 gm. of—		
	Silage juice.	Silage containing moisture.	Silage on dry basis.	Silage juice.	Silage containing moisture.	Silage on dry basis.	Silage juice.	Silage containing moisture.	Silage on dry basis.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Acetic	0.599	0.455	1.897	0.378	0.266	0.881	0.886	0.675	2.836
Propionic	.043	.033	.137	.020	.014	.046	.064	.049	.205
Total volatile	.642	.488	2.034	.398	.280	.927	.950	.724	3.041
Lactic	1.902	1.446	6.02	1.746	1.229	4.07	1.474	1.123	4.72
Total acidity	2.544	1.934	8.054	2.144	1.509	4.997	2.424	1.847	7.761

Acid.	OAT AND PEA SILAGE (PEAS, 75 PER CENT: OATS, 25 PER CENT, ON DRY BASIS). (Moisture, 74 per cent; dry material, 26 per cent.) Acidity in 100 gm. of —			OAT AND PEA SILAGE (PEAS, 50 PER CENT: OATS, 50 PER CENT, DRY BASIS). (Moisture, 74.3 per cent; dry material 25.7 per cent.) Acidity in 100 gm. of —			CLOVER SILAGE. (Moisture, 75 per cent; dry material, 25 per cent.) Acidity in 100 gm. of —		
	Silage juice.	Silage containing moisture.	Silage on dry basis.	Silage juice.	Silage containing moisture.	Silage on dry basis.	Silage juice.	Silage containing moisture.	Silage on dry basis.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Acetic	0.760	0.562	2.148	0.681	0.506	1.969	0.9	0.675	2.70
Propionic	.054	.040	.154	.064	.048	.184	.056	.042	.168
Total volatile	.814	.602	2.302	.745	.554	2.153	.956	.717	2.868
Lactic	1.772	1.311	5.01	1.592	1.183	4.602	.860	.645	2.580
Total acidity	2.586	1.913	7.312	2.337	1.737	6.755	1.816	1.362	5.448

Acid.	CLOVER AND WHEAT-STRAW SILAGE (CLOVER, 75 PER CENT: STRAW, 25 PER CENT). (Moisture, 73.5 per cent; dry material, 26.5 per cent.) Acidity in 100 gm. of—			CLOVER AND WHEAT-STRAW SILAGE (CLOVER, 50 PER CENT: STRAW, 50 PER CENT). (Moisture, 75 per cent; dry material, 25 per cent.) Acidity in 100 gm. of—			ALFALFA SILAGE. (Sample taken 3 months after siloing; moisture, 75 per cent; dry, material 25 per cent.) Acidity in 100 gm. of—		
	Silage juice.	Silage containing moisture.	Silage on dry basis.	Silage juice.	Silage containing moisture.	Silage on dry basis.	Silage juice.	Silage containing moisture.	Silage on dry basis.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Acetic	0.884	0.650	2.450	0.674	0.506	2.028	1.253	0.940	3.76
Propionic	.064	.047	.179	.063	.047	.188	.153	.115	.46
Total volatile	.948	.697	2.629	.737	.553	2.215	1.406	1.055	4.22
Lactic	.966	.710	2.870	.927	.695	2.781	Trace.	Trace.	Trace.
Total acidity	1.914	1.407	5.499	1.664	1.248	4.997	1.406	1.055	4.22

TABLE III.—*Acidity produced by pea silage, oat silage, oat and pea silage, clover silage, clover and wheat-straw silage, alfalfa silage, alfalfa and wheat-straw silage in small silos, Idaho, 1916*—Continued

Acid.	ALFALFA AND WHEAT-STRAW SILAGE (ALFALFA, 75 PER CENT; STRAW, 25 PER CENT). (Moisture, 75 per cent; dry material, 25 per cent.)			ALFALFA AND WHEAT-STRAW SILAGE (ALFALFA, 50 PER CENT; STRAW, 50 PER CENT). (Moisture, 75.5 per cent; dry material, 24.5 per cent.)		
	Acidity in 100 gm. of—			Acidity in 100 gm. of—		
	Silage juice.	Silage containing moisture.	Silage on dry basis.	Silage juice.	Silage containing moisture.	Silage on dry basis.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Acetic	0. 458	0. 344	1. 375	0. 646	0. 488	1. 992
Propionic	. 328	. 246	. 983	. 058	. 044	. 180
Butyric	. 724	. 543	2. 172	. 389	. 294	1. 198
Total volatile	1. 510	1. 133	4. 530	1. 093	. 826	3. 370
Lactic	. 000	. 000	. 000	. 000	. 000	. 000
Total acidity	1. 510	1. 133	4. 530	1. 093	. 826	3. 370

PEA SILAGE

When opened, the pea silage had a very pleasant odor, but was a little dark in color. The determination of acids showed that a fermentation had taken place which was similar to that found in normal corn silage (Table III).

OAT SILAGE

The oat silage had a very fine color and odor. Dairy stock ate it with relish. The acid fermentation was normal and similar to that in corn silage (Table III).

OAT AND PEA SILAGE

The three combinations of oats and peas made excellent silage from the standpoint of acid fermentation. In every instance, the silage had a good color and odor, and the stock ate it readily. In so far, then, as the acid fermentation is concerned, the proportion of the two crops used makes practically no difference. This fact is of considerable importance to the man who might wish to grow the greatest tonnage possible per acre by comparatively heavy sowings of peas (Table III).

CLOVER SILAGE

The clover silage was sampled four months after filling the silo. The silage was quite dark in color, but had an agreeable odor. The acid fermentation was similar in the kind of acids developed, but the proportion of volatile ands nonvolatile acid varied from that found in normal corn silage. The dairy stock ate this silage with relish (Table III).

CLOVER AND WHEAT-STRAW SILAGE

The appearance of clover and clover and wheat straw mixture was very similar. Both kinds of silage kept well, and practically no difference in color or odor could be noted. It appeared that dairy stock ate the clover and wheat-straw silage with more relish, but both kinds were entirely consumed at each feeding. The acid fermentation in both cases was similar to that in corn silage. Siloing a portion of wheat straw with clover offers an opportunity for securing the maximum food value from wheat straw that otherwise is generally burned in the stack (Table III).

ALFALFA SILAGE

In a sample of silage made from alfalfa alone taken three months after siloing no butyric acid was found. Moreover, lactic acid was absent. A sample taken nine months after siloing had a strong odor of butyric acid. It was examined qualitatively by the orientation tests suggested by Dyer, and the presence of butyric acid was confirmed. The silage was unfit for feeding purposes. This fact showed that alfalfa silage gradually deteriorates with age and confirms the conclusions of Reed and Fitch (*11*), who state that alfalfa can be made into silage if it is fed soon after siloing, but on standing it rapidly becomes unfit for feeding purposes. Experiments are now under way to determine the practicability of adding crude glucose to alfalfa when siloed for the purpose of raising the percentage of fermentable carbohydrates, the object being to insure an acid fermentation that is similar to that in corn silage.

Reed and Fitch state that—

> There is as much acid produced in alfalfa silage as in kafir or cane silage. This would indicate that the acid content of silage is not always an index to the quality of silage.

In the author's opinion the criterion of good silage is the kind of acids present rather than the quantity. Good silage must have a sufficient quantity to insure its keeping, but beyond this point silage may vary in amount of acidity and yet be classed as normal silage. All good silage examined by the writer contained lactic, acetic, and propionic acids, and in almost all cases lactic acid was in excess of the sum of acetic and propionic acids. It is assumed that no determinations were made by Reed and Fitch on the kinds of acids present in alfalfa silage, but that all the acidity was calculated in terms of lactic acid. Obviously, by this method it would appear to them that quantity of acids was not a determining factor.

The author found that in alfalfa and other silage of poor quality butyric acid was always present in amounts varying with the degree of spoiling that it had undergone. Moreover, in all samples of alfalfa silage lactic acid was found only in traces. It is hard to explain why lactic acid is absent in alfalfa silage, when it is usually the predominating

acid in good silage. There is a possibility that alfalfa, lacking sufficient carbohydrate, first develops lactic acid, which is in turn reduced to propionic acid and by further reductions to butyric acid by microorganisms. Such reactions have been shown by Fitz (*8*) to be possible by certain organisms. However, no study has been made of the acids of alfalfa silage developed during the first few days after siloing, which is necessary to determine this point. The suggestion, therefore, is only tentative. It is also possible that some butyric acid develops at the expense of the proteins. Therefore poor silage may actually contain as much acid as a silage of good quality, but may, nevertheless, be unfit for feeding purposes.

ALFALFA AND WHEAT-STRAW SILAGE

The alfalfa and wheat straw combinations did not make good silage. The silage had a very disagreeable odor and was not fit for feeding purposes. A glance at Table III shows that the acid fermentation differed from the fermentation that takes place in good silage.

TABLE IV.—*Summary of volatile and nonvolatile acids in silage made from forage crops in small silos*

[Results are given on 100 gm. of silage, dry basis]

Silage.	Acetic acid.	Propionic acid.	Butyric and volatile acids.	Total volatile acids.	Lactic acid.	Total acidity.
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Alfalfa	3.76	0.469	....	4.229	0.000	4.229
Alfalfa 75 per cent; Wheat straw 25 per cent	1.375	.983	2.172	4.53	.000	4.53
Alfalfa 50 per cent; Wheat straw 50 per cent	1.992	.180	1.198	3.37	.000	3.37
Clover	2.70	.168	....	2.868	2.580	5.448
Clover 75 per cent; Wheat straw 25 per cent	2.45	.179	....	2.629	2.870	5.499
Clover 50 per cent; Wheat straw 50 per cent	2.028	.188	....	2.216	2.781	4.997
Peas	1.897	.137	....	2.034	6.02	8.054
Peas 87½ per cent; Oats 12½ per cent	2.836	.205	....	3.041	4.72	7.761
Peas 75 per cent; Oats 25 per cent	2.148	.154	....	2.302	5.01	7.312
Peas 50 per cent; Oats 50 per cent	1.969	.184	....	2.153	4.602	6.755
Oats	.881	.046	....	.927	4.07	4.997

SUMMARY

(1) Previous investigations showed that all samples of high-class corn silage contain lactic, acetic, and propionic acids, the nonvolatile lactic acid usually occurring in excess of the sum of the volatile, acetic, and propionic acids. Of the volatile acids, acetic is greatly in excess of the propionic acid.

(2) The crops and crop mixtures under examination which showed an acid fermentation similar to corn silage and were all first-class silage are as follows:

Oats and peas in any proportion.	Wheat and peas.
Oats.	Clover.
Peas.	Clover and wheat straw.

(3) Crops and crop mixtures under examination which did not develop an acid fermentation similar to corn, and were unfit for feeding purposes are as follows:

Alfalfa, unless fed soon after siloing.
Alfalfa and wheat straw.

(4) Butyric acid was always found in samples of spoiled or partly spoiled silage.

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PIÑON BLISTER-RUST

By GEORGE G. HEDGCOCK and ELLSWORTH BETHEL, *Pathologists*, and N. REX HUNT, *Assistant Pathologist, Forest Pathology Investigations, Bureau of Plant Industry, United States Department of Agriculture*[1]

INTRODUCTION

A species of Cronartium on *Ribes aureum* has been known in Colorado for many years, having been collected by one of the writers (Bethel) in the Ute Indian Reservation in southwestern Colorado in 1897, and in 1909 at Boulder and Denver, and various other places since that time.[2]

Since this species of Cronartium closely resembles *Cronartium ribicola* Fischer de Wald, the cause of the white-pine blister-rust, and likewise attacks species of Ribes, and because no species of caulicolous Peridermium has hitherto been known in Colorado which could serve as its æcial stage, it has been considered by some to be identical with *C. ribicola*. On the other hand, the occurrence of this rust at Boulder, Colo., on *Ribes aureum* for several years in close proximity to several white pines (*Pinus strobus*) which remained uninfected indicated that it was not *C. ribicola*.

The earliest collection of this western species of Cronartium is now believed to be one of the uredinial stage collected by Bartholomew and reported by Arthur[3] (*2, p. 130*) in 1892 in Kansas. This was at first identified by Arthur (*7, p. 63*) as presumably *Cronartium ribicola* Arthur, upon receiving data and specimens of Mr. Bethel's collections in 1909, wrote him as follows:[4]

> The Cronartium on Ribes from Boulder is an interesting species. I think you are right in regarding it as an undescribed form, and this accounts for the uredo on Ribes found in Kansas, which is reported under *Cronartium ribicola* in the North American Flora (*1*).

Spaulding (*6*) in 1911 referred to the Kansas collection as *Cronartium ribicola*, basing his action on Arthur's first determination (7). Later, on seeing a specimen of the collection in Kansas by Bartholomew, Spaulding considered it a distinct species. Arthur and Kern (*2*), in 1914,

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[1] The writers are under obligations to Mr. F. V. Coville, of the office of Economic and Systematic Botany, Bureau of Plant Industry, for the determination of species of Ribes; to the State Historical and Natural History Society, Denver, Colo., for office room and other courtesies; to the officers of District 2, Forest Service, Denver, for cooperation in securing information regarding forest conditions; and to Mr. Hugh McGeary and Mrs. S. N. Keith, Bayfield, Colo., and Mr. R. Branson, Mancos, Colo., for material assistance in expediting the work of investigation.

[2] As shown by correspondence in the files of this Office, Mr. Bethel made collections at other places in Colorado previous to 1909, but the material is stored and not available for examination. No localities are listed in this paper unless the specimens collected have been recently examined by the writers.

[3] Reference is made by number (italic) to "Literature cited," pp. 423–424.

[4] Letter to Mr. Bethel dated Nov. 11, 1909.

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treated this species under *C. ribicola*, but stated that its occurrence on *Ribes* (*longiflorum*) *aureum* in the parks of Denver and Boulder was unexplainable. That this rust is not *C. ribicola* was predicted by Hedgcock and Long (4) in 1915 and by Bethel in 1917 in North American Uredinales No. 1609.

In connection with the white-pine blister-rust investigations[1] during the summer of 1917, a thorough investigation of the rust was begun. The species of caulicolous Peridermiums previously known in Colorado and Arizona are *Peridermium pyriforme* Peck, *P. filamentosum* Peck, and *P. harknessii* Moore, all of which attack *Pinus ponderosa* and *P. contorta*, and whose telial stages are well known. The first problem, therefore, was to find a species of Peridermium which could be shown to be the æcial stage of this species of Cronartium. Since the northern and central parts of the State had long been a favorite collecting ground of mycologists and no such species of Peridermium had been discovered, it seemed probable that it would be found in the southern part of the State, where less collecting had been done. The first collection of this species of Cronartium had been made in the Ute Indian Reservation along Los Pinos River near Bayfield, about 20 miles north of the Colorado-New Mexico boundary; so this region was surveyed first.

On May 25, 1917, the senior writer found a Peridermium, apparently a new species, on *Pinus edulis* south of Bayfield. The following day two of the writers (Hedgcock and Bethel) found the telia of the Cronartium on fallen leaves of *Ribes aureum* north of Bayfield, near the spot where Mr. Bethel had collected the fungus in 1897. Culture material of the species of Peridermium was sent to Washington, D. C., where another of the writers (Hunt) made inoculations with æciospores on species of Castilleja, Comandra, and Ribes, with the result that *R. odoratum* became infected with the species of Cronartium, establishing the new species of Peridermium as the æcial stage of the western species of Cronartium. Repeated inoculations given elsewhere in this paper have fully corroborated this result.

This species of Cronartium was first found in Arizona by Mr. Goodding on October 2 at Prescott, Ariz., and was at first supposed to be *C. ribicola*. Dr. Long, assisted by Messrs. Goodding and Llewellyn, made a thorough survey of the region around Prescott in October and found this species of Cronartium abundant on both cultivated and wild plants of *Ribes* spp. in several localities, some widely separated, but did not find the æcial form on pines. On October 28 one of the writers (Bethel) found one tree of piñon (*Pinus monophylla*) near Prescott dis-

[1] The work in Colorado was in charge of Dr. G. G. Hedgcock, assisted by Messrs. E. Bethel, N. R. Hunt, E. B. Payson, E. L. Johnson, R. Thompson, and H. L. Gaymon. Dr. W. H. Long was in charge in New Mexico and Arizona, assisted by Messrs. C. S. Lewellyn and L. N. Goodding. Mr. A. S. Rhoads assisted in the work of inoculation at Washington, D. C. The work in Colorado was in cooperation with the Colorado Agricultural Experiment Station, which was in charge of the inspection of nursery stock and nurseries for the white-pine blister-rust.

eased with the æcial form, then past maturity. Old æciospores were found to be present by Messrs. Hedgcock and Bethel. This tree was examined by Messrs. Bethel and Hunt in May, 1918, but new, fresh æcia had not been formed. Old æciospores were still present. Messrs. Hedgcock and Bethel collected specimens of the *Cronartium* sp. at several points around Prescott during the latter part of October, 1917, and pronounced it distinct from *C. ribicola*, but identical with the species of Cronartium previously found at Bayfield and other localities in Colorado.

DESCRIPTION OF THE FUNGUS

The morphology of this new species of Peridermium differs so much from that of *P. strobi* Kleb., the æcial stage of *Cronartium ribicola*, that the writers are led to believe it is the æcial stage of a distinct species. The Colorado species of Cronartium on *Ribes* spp. is here designated "*Cronartium occidentale*," with the following description:

Cronartium occidentale, n. sp. (Pl. 54, 56, 57).

O.—Pycnia caulicolous, scattered, forming blisters 0.5 cm. or more in diameter; exudate orange-chrome;[1] pycniospores hyalin, pyriform or obovoid to ellipsoid, 2 to 3 by 3 to 5 μ, averaging 2 by 4 μ; pycnial scars seldom found (Pl. 56).

I.—Æcia caulicolous, sometimes causing slight hypertrophy; æcial cavities large, often entirely hidden by the bark; peridia inconspicuous, thin, evanescent, only slightly protruding, if at all, rupturing at irregular cracks in the bark or near the top if protruding; peridial cells variable, nearly smooth on outer surface, verrucose on inner surface, 12 to 26 by 17 to 36 μ, averaging 19 by 25 μ, walls 1 to 5 μ thick; æciospores variable, usually obovoid to ellipsoid, 12 to 28 by 22 to 38 μ, walls colorless, 1 to 5 μ thick, averaging 3 μ, outer wall coarsely verrucose, with deciduous tubercules, which in end view are 0.7 to 2.5 by 1 to 4.6 μ, averaging 1.4 by 1.8 μ, in side view 1.7 to 4.2 μ, averaging 2.7 μ long (Pl. 54).

In Arizona on *Pinus monophylla*.

In Colorado on *Pinus edulis*.

The type specimen of the æcial stage is FP 26227[2] on *Pinus edulis*, collected by Messrs. E. Bethel and H. L. Gaymon at Bayfield, Colo., on July 13, 1917.

II.—Uredinia hypophyllous, rarely amphigenous, scattered on irregularly-rounded areas; sori light yellow to yellow, pustular, 0.2 to 2 mm. in diameter, dehiscent by a central opening; urediniospores 13.5 to 20 by 18.5 to 32 μ, averaging 16 by 24 μ, with colorless walls 2 to 3 μ thick, sharply echinulate on outer surface.

III.—Telial columns hypophyllous or rarely amphigenous, and occasionally on the petioles and peduncles, cylindrical or nearly so, 60 to 170 μ thick, up to 4 mm. long, walnut-brown to Vandyke-brown; teliospores oblong to cylindric, 9 to 19 by 27 to 56 μ; wall nearly colorless, 0.4 to 2 μ thick (Pl. 57).

In Arizona on *Ribes aureum*, *R. odoratum*, and *Grossularia reclinata*×*G. hirtella*.[3]

In Colorado on *Ribes aureum*, *R. inebrians*, and *Grossularia leptantha*.

In Washington, D. C., by cultures on *Ribes americanum*, *R. aureum*, *R. coloradense*, *R. giraldi*, *R. glandulosum*, *R. malvaceum*, *R. nigrum*, *R. odoratum*, *R. sanguineum*, *R. sp.*, *Grossularia inermis*, *G. missouriensis*, and *G. reclinata*×*G. hirtella*.

[1] Color terms used are from RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 pl. (col.). Washington, 1912.

[2] The type specimens of *Cronartium occidentale* are deposited in the Pathological Collections of the United States Department of Agriculture at Washington, D. C.

[3] The type specimen of the uredinial and telial stages is FP 24420 on *Ribes aureum* collected by Messrs. Bethel and Hunt at Bayfield, Colo., on September 15, 1917.

For the convenience of those who wish to compare *Cronartium occidentale* with *C. ribicola* the principal points of difference are shown below in parallel columns. The characteristics of *C. ribicola* are furnished by Dr. Perley Spaulding and Dr. R. H. Colley, of this Office.

Cronartium occidentale differs from *C. ribicola* in its pycnial and æcial stages, in its æcial hosts, and in its behavior on some telial hosts as follows:

CRONARTIUM OCCIDENTALE	CRONARTIUM RIBICOLA
O.—PYCNIAL EXUDATE orange chrome; PYCNIAL SPOTS uncommon and not conspicuous.	O.—PYCNIAL EXUDATE honey-yellow; PYCNIAL SPOTS numerous and conspicuous.
I.—ÆCIAL AREAS showing slight, if any hypertrophy (Pl. 54). ÆCIOSPORES borne in large cavities, peridia seldom prominent, often not visible (Pl. 54). PERIDIUM, thin, evanescent; SPORES usually released through irregular cracks in the overlying bark; SPORES 12 to 28 by 22 to 38 μ; wall 1 to 5 μ, averaging 3.0 μ thick.	I.—ÆCIAL AREAS usually showing a marked fusiform swelling, especially on younger trees (Pl. 55). ÆCIOSPORES borne within closely aggrerated, prominently protruding peridia (Pl. 55). PERIDIUM, thick, persistent; SPORES released by irregular breaking of the peridia. SPORES 18 to 20 by 22 to 33 μ, wall 2.0 to 2.5 μ thick.
O and I.—On piñon (1- and 2-needled) pines.	O and I.—On white (5-needled) pines.
I and II.—Incubation period on *Ribes* spp. 12 to 36 days.	I and II.—Incubation period on *Ribes* spp. 5 to 14 days.
II and III.—*Ribes nigrum*, one of the poorest hosts in inoculations. *Grossularia leptantha* best host in at least one locality in Colorado.	II and III.—*Ribes nigrum* best host. *Grossularia leptantha* poorest host in inoculations.
Native of America.	Native of Old World.

The striking differences in the appearance and morphology of the æcial stages of *Peridermium occidentale* and *Peridermium strobi* might be thought to be due, in part at least, to differences in the bark of the hosts, since *Pinus edulis* has a rather thick bark while *Pinus strobus* and other white pines have a thin bark. However, *Peridermium harknessii*, which sloughs off the overlying bark tissues in much the same way as *Peridermium occidentale*, has the same characteristics whether found on the thin-barked *Pinus contorta* or the thick-barked *Pinus ponderosa*. *Peridermium pyriforme*, which produces numerous protruding peridia much like those of *Peridermium strobi*, also occurs on *Pinus contorta* and on *Pinus ponderosa*, with no apparent modification resulting from the variation in the thickness of the bark. *Peridermium filamentosum* on the same hosts behaves similarly.

Although it was soon evident that the incubation period on *Ribes* spp. was longer for *Cronartium occidentale* than for *C. ribicola*, only one test has been made for the specific purpose of comparison on this point. On November 14 four very similar plants of *Grossularia inermis*

and two of *Ribes odoratum* were selected from the stock beds. One half of these plants were inoculated with *C. occidentale* by Hunt and the others with *C. ribicola* by Mr. G. F. Gravatt, of this Office. All plants were covered with bell jars and put under a side bench of the greenhouse. A tight glass and wood partition separated the two lots of plants, but the most widely separated plants were less than 6 feet apart.

On November 21 the plants of *Grossularis inermis* inoculated with *Cronartium ribicola* were producing urediniospores. On November 22, urediniospores were abundant on these plants, and some were being produced by part of the numerous uredinia on the plant of *Ribes odoratum*. A careful examination with a hand lens failed to reveal any uredinia in process of formation on the plants inoculated with *C. occidentale*, although these plants later developed a heavy infection, first producing a few spores on November 27. Abundant uredinia and telia were produced on all of the plants inoculated. The difference in the length of the incubation period is variable but marked, and this fact supports the view that the two forms are distinct species.

To summarize briefly, *Cronartium occidentale* differs essentially from *C. ribicola* as follows: In the Peridermium or æcial stage the former bears its thicker-walled æciospores in a few large cavities under the bark and bordered with thin and evanescent peridia, while those of the latter are borne in numerous small cavities beneath thicker-walled, protruding, persistent peridia; the pycnial stage of the former has an orange-chrome pycnial exudate, the latter a honey-yellow one; the incubation period from the æcial stage to the uredinial stage is longer in the former than in the latter; the telial stage of the former infects *Grossularia leptantha* abundantly and *Ribes nigrum* rarely and sparsely, while the reverse is true in the case of the latter.

DISTRIBUTION OF CRONARTIUM OCCIDENTALE

DISTRIBUTION OF THE ÆCIAL STAGE

The æcial stage is here designated "*Peridermium occidentale*" to distinguish it from the æcial stages of other species of the form-genus Peridermium. It has been found only in two States, Arizona and Colorado (fig. 1), as follows:

ARIZONA:

On *Pinus monophylla*.—Near **Prescott**,[1] HEDGCOCK and BETHEL, October 28.[2]

COLORADO:

On *Pinus edulis*.—**Bayfield**, HEDGCOCK and BETHEL, May 25 (first collection of æcial stage), BETHEL and GAYMON, June 23, 28, and 30, July 12 and 13, BETHEL, August 26 and 31; **Cedar Creek**, Montrose County, BETHEL, July 31; **Glenwood Springs**, PAYSON, August 31, and HUNT and BETHEL, October 3; **Mancos**, BETHEL and PAYSON, August 16, BETHEL, August 17, and BETHEL and HUNT, September 19; **Mesa Verde National Park**, BETHEL and HUNT, September 21.

[1] All data on distribution are supported by numbered specimens in our collection.
[2] For data with the year omitted refer to the year 1917.

DISTRIBUTION OF THE UREDINIAL AND TELIAL STAGES

The uredinial and telial stages have been collected as follows:

Arizona:[1]

On *Ribes aureum.*—**Copper Basin,** Long, October 6; **Cottonwood,** Hedgcock and Bethel, October 31; **Prescott,** Gooding, September 27, Long and Gooding, October 6, Long, October 6, Hedgcock, Long, Bethel, and Llewellyn, October 26 and 27, and Hedgcock and Bethel, October 29; **Walnut Creek,** Llewellyn, October; **Verde River Valley,** Llewellyn, October.

On *Ribes odoratum.*—**Prescott,** Gooding, October 2, Long and Gooding, October 6, Long, October 6 and 11, and Hedgcock and Bethel, October 29.

On *Grossularia reclinata* × *G. hirtella.*—**Prescott,** Long and Gooding, October 6, Long, October 10, and Hedgcock, Long, Bethel, and Llewellyn, October 26 and 27.

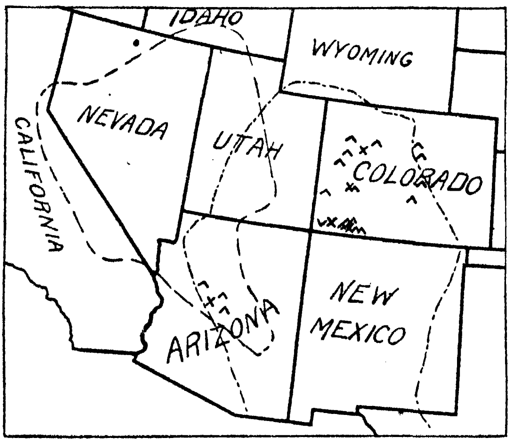

Fig. 1.—Outline sketch map showing the distribution of *Pinus edulis* and of *P. monophylla* in the United States. The former is included in the broken line of dashes and dots to the right, and the latter by the line of dashes to the left. In this region is the known distribution of *Cronartium occidentale*. Localities where collections of the different stages of the fungus have been made are indicated on the map as follows: V, æcial stage on species of pine; Λ, uredinial and telial stages on species of Ribes: ×, all forms present.

Colorado:

On *Ribes aureum.*—**Boulder,** Bethel, August, 1909, August 1, 1911, September, 1913, July 1, August 1, and August 4, 1914, Hedgcock, October 7 and 17, 1914, October 13, 1916; **Bayfield,** Bethel, August 3, 26, and September 12; **Canon City,** Hunt and Bethel, October 5; **Cedar Creek,** W. W. Robbins, October, 1912, and Bethel, July 9; **Colorado Springs,** Hunt and Bethel, October 13; **Cimarron,** Hedgcock, October 10 and 11; **Denver,** Bethel and Hunt, October 10 and 16, Hedgcock and Bethel, October 17, Hedgcock and Thomson, August 11, Hedgcock, October 17, 1914, October 11, 1916, September 25; **Devils Creek,** Bethel and Payson, August 11, Bethel, August 26 and 27, Bethel and Hunt, September 8 and 24; **Debeque,** Hedgcock, October 16; **Durango,** Bethel and Hunt, September 17; **Florida River,** Bethel and Hunt, September 15; **Glenwood Springs,** Hedgcock, Bethel and Hunt, October 4; **Hermosa,** Bethel and Hunt, September 16; **Los Pinos River,** Bethel, July, 1897 (first collection of telial stage); **Mancos,** Bethel and Payson, August 16, and Bethel and Hunt, September 22; **McCoy,** Hedgcock, September 28; **Meeker,** Hedgcock, October 2; **Naturita,** Payson, July 24 and August 18; **Piedra,** Bethel and Payson, August 11; **Rifle,** Hedgcock, October 3, 5, and 6.

On *Ribes inebrians.*—**Glenwood Springs,** Payson, August 31.

On *Grossularia leptantha.*—**Cimarron,** Hedgcock, October 11; **Glenwood Springs,** Payson, August 29, Bethel and Hunt, October 3, and Hedgcock, October 4.

[1] All Arizona collections were made in Yavapai County.

DISTRICT OF COLUMBIA, WASHINGTON;[1]

On *Ribes americanum*.—HUNT, November 11:

On *Bibes aureum*.—HEDGCOCK, August 31, September 8, 19, and 21, and HUNT, November 3.

On *Ribes giraldi*.—HEDGCOCK, September 21, and HUNT, November 3.

On *Ribes malvaceum*.—HEDGCOCK, September 11, and HUNT, November 3.

On *Ribes nigrum*.—HUNT, November 3.

On *Ribes* sp.—(near *R. aureum*) HEDGCOCK, September 21.

KANSAS:

On *Ribes aureum*.—Rooks County, E. BARTHOLOMEW, August 22, 1892. (First collection of uredinial stage.)

Although the survey for *Peridermium occidentale* was continued in Colorado throughout the summer of 1917 until late in October, only 42 trees of *Pinus edulis* were found diseased by it. These were found in five widely separated localities. In Arizona only one diseased tree of *Pinus monophylla* was found late in the season. The finding of telia on *Ribes aureum* in Arizona in the vicinity of trees of *Pinus cembroides* indicates that this also may be an æcial host. No survey was made of regions where *Pinus quadrifolia* is found. These four species of pine are known as piñon pines. Since this rust is the only one known to occur on the stems of piñons, and is, so far as known, confined to piñons, it is called the "piñon blister-rust."

Peridermium occidentale has not been found on the native white pines *Pinus aristata* and *P. flexilis*, although both species occur frequently in association with *Grossularia leptantha* and *Ribes inebrians*, which are native hosts for the Cronartium. These species of Grossulariaceae often range from the piñon belt, where *P. edulis* abounds, to the higher altitudes, where the white pines occur, furnishing a means for spreading the rust in the uredinial and telial stage from the former species of pine to the latter if they were susceptible.

Cronartium occidentale in the uredinial and telial stages was collected in 1917 in 18 localities in Colorado and 5 in Arizona. Although all of these collections except those at Denver and Boulder, Colo., were made in regions where piñon pines occur, the Peridermium stage was collected only in 6 localities. *Ribes aureum* is the principal host for the uredinial and telial stages in nature and is usually heavily infected under favorable conditions, especially along streams and under moist conditions. At Glenwood Springs, Colo., *Grossularia leptantha*, growing on dry hillsides was abundantly infected, while *R. aureum* and *R. inebrians* were found infected in only one locality for each, and of the latter species only one clump or bush was infected. *R. odoratum* has been planted in many gardens in and around Prescott, Ariz. This species is doubtfully distinct from *R. aureum*, is equally susceptible to the fungus, and was abundantly infected at Prescott. Cultivated currants (*Ribes* spp.) and

[1] All collections from the District of Columbia are from artificial inoculations made in the greenhouse at Washington by Messrs. Hedgcock, Hunt, and Rhoads.

gooseberries were frequently found in Colorado in regions near badly diseased plants of *R. aureum*, but were never found infected. At Prescott a few plants of cultivated gooseberries (*Grossularia reclinata* × *G. hirtella*) were found to be slightly infected.

The apparent rarity of *Peridermium occidentale* is perhaps due chiefly to the fact that plants of susceptible Ribes are not of common occurrence in piñon forests. In localities at 8,000 feet or more in elevation, as in the San Luis Valley in southern Colorado, *Grossularia leptantha* is common among piñons, but lower down on the piñon mesas no species of Ribes are found except along the streams. Here *R. aureum* occurs frequently. In such localities agricultural activities have removed most of the trees of *Pinus edulis*, which formerly grew there in abundance.

The piñons of Colorado are all of the 2-leaved species, *Pinus edulis*, and are found almost exclusively west of the 106th Meridian and south of the 39th Parallel (fig. 1). They occur at an altitude of 5,000 feet to 8,500 feet, although in exceptional cases, as on Marshall Pass, they ascend to nearly 10,000 feet. They cover an area of more than 40,000 square miles of the State, chiefly the mesas (low table-lands) in isolated areas. Only a small part of this region was surveyed during 1917, owing to the lack of time and because of the difficulty of access, since there are few railroads or automobile roads in this section.

INOCULATIONS WITH CRONARTIUM OCCIDENTALE

INOCULATIONS WITH ÆCIOSPORES

The first inoculations with the æciospores of *Peridermium occidentale* from *Pinus edulis* were made at the pathological greenhouses at Washington, D. C., on June 1, 1917, with material (FP 24667) collected on May 25. Plants of *Castilleja linearifolia*, *Comandra umbellata*, and *Ribes odoratum* were used. On June 14 one of the plants of *R. odoratum* was found to be infected with a species of Cronartium. Two more *R. odoratum* plants were immediately inoculated with spores from the same lot of æcial material. On June 25 abundant uredinia were observed to be forming on several leaves of both plants. Successful inoculations were also made under rigid quarantine conditions in a unit of the quarantine house of the Federal Horticultural Board. Other æciospore inoculations were made at various times with material from different collections and localities. Inoculated plants of *R. aureum*, *R. odoratum*, and of *Grossularia inermis*, and one plant of *R. americanum* became infected, but those of *R. nigrum* and *G. missouriensis* remained uninfected. Control plants of all species were uninfected. The last successful æciospore inoculation was made on October 2, 1917, with material collected on September 21. No infections resulted where the æciospores used had been collected and kept for more than 20 days. Table I gives a summary of the results of the inoculations with æciospores and urediniospores at Washington, D. C.

TABLE I.—*Inoculations with æciospores and urediniospores of Cronartium occidentale at Washington, D. C.*

Host species.	Æciospore inoculations.			Urediniospore inoculations.		
	Plants inoculated.	Plants infected.	Plants uninfected.	Plants inoculated.	Plants infected.	Plants uninfected.
Ribes americanum [a]	4	1	3	9	5	4
Ribes aureum	6	3	3	8	4	4
Ribes coloradense	0	0	0	2	2	0
Ribes giraldi	0	0	0	1	1	0
Ribes glandulosum	1	0	1	2	1	1
Ribes malvaceum	0	0	0	2	1	1
Ribes nigrum [a]	15	0	15	22	2	20
Ribes odoratum [b]	32	16	16	45	28	17
Ribes sanguineum	0	0	0	1	0	1
Ribes sp. (?)	15	0	15	8	3	5
Grossularia inermis	10	3	7	10	5	5
Grossularia missouriensis	5	0	5	7	1	6
Grossularia reclinata [c]	0	0	0	1	0	1
Total	88	23	65	118	53	65

a Plants of *R. americanum* and *R. nigrum* inoculated with uredionispores were showered daily with spores for several days.
b R. odoratum was used almost exclusively when conditions were unfavorable; hence the percentage of uninfected plants of this species is misleading.
c The plants of *G. reclinata* were apparently a hybrid between this species and *G. hirtella*.

In addition to the inoculations made in the greenhouses at Washington, a number of plants of *Ribes aureum* were inoculated in the open at Denver and at Bayfield, Colo. Nearly all of these were successful, uredinia and telia being produced in abundance in most cases until in late October, when the heavy frosts came. Attempts to inoculate *R. americanum* and garden currants and gooseberries in the open at Denver and at Bayfield met with failure.

INOCULATIONS WITH UREDINIOSPORES

At Washington, D. C., inoculations with urediniospores were successful on *Ribes americanum*, *R. aureum*, *R. coloradense*, *R. giraldi*, *R. glandulosum*, *R. malvaceum*, *R. nigrum*, *R. odoratum*, *Ribes* sp., *Grossularia inermis*, *G. missouriensis*, and *G. reclinata*×*G. hirtella*. On plants of the *R. aureum* type many of the infected areas enlarge rapidly on the leaves, and the infection often spreads from leaf to leaf on the same and adjoining healthy plants. The uredinia in heavy infections are borne close together and eject spores in such masses that the leaf tissue appears to be covered. On plants of *R. malvaceum*, of *G. inermis*, and of *G. missouriensis* infected areas enlarge rather slowly and do not usually produce uredinia or spores in abundance. The fungus sometimes spreads to new areas of diseased leaves and even to new leaves on infected plants of *G. inermis*, but has not been known to do so on plants of *R. malvaceum* or of *G. missouriensis*. No plants *of R. americanum* or *R. nigrum*

became infected until late in the summer, although many inoculations were attempted. On plants of these two species the infected areas developed slowly until they became from 2 to 4 mm. in diameter. The invaded tissues often die soon after infection. Uredinia are scanty if produced at all, and few spores develop. The incubation period for these two species may be as long as 36 days, although 15 days are the maximum for other species. The disease has not been observed to spread to new areas or to new leaves on plants of these species.

The leaves of many plants were carefully examined with a hand lens almost daily, beginning 5 or 6 days after the date of inoculation. In no case were uredinia found forming in less than 10 days following inoculations with æciospores, nor in less than 9 days following inoculations with urediniospores. Urediniospores are normally produced in 12 to 15 days after inoculations with either form of spores, except as noted above for *R. americanum* and *R. nigrum*.

A summary of inoculations with urediniospores during 1917 and the spring of 1918 at Washington, D. C., is given in Table I. Urediniospores and teliospores were produced throughout the winter, successive inoculations being made every six weeks.

INOCULATIONS WITH TELIOSPORES

The first inoculations with the telia of *Cronartium occidentale* from *Ribes aureum* sent in from Colorado were made in 1914. Others were made in 1916. The material used in these inoculations was in such condition that probably no viable teliospores were present, as no results have been obtained. In 1917 about 4,000 inoculations were made on 23 species of pine. The larger part of these were made in the field, in Colorado and Arizona, but a considerable number were made in the greenhouse at Washington, D. C. It is hoped that some indication of the results may be obtained during 1918 either through the development of pycnia or by determining the presence of the mycelium of the species of Cronartium by Colley's method (*3*).

DISSEMINATION OF CRONARTIUM OCCIDENTALE

Cronartium occidentale, like other species of the genus Cronartium in the United States (*4*, *6*), is disseminated in three spore forms: Æciospores, urediniospores, and teliospores.

Although the æcia of the Peridermium form of this fungus on *Pinus edulis* were first found in the fruiting stage on May 25, they may sometimes mature at a somewhat earlier date. Viable spores of the Peridermium were found in specimens collected in southern Colorado as late as September 21. The æciospores are discharged slowly, owing to the fact that they are held under the bark in large flattened cavities which rupture by means of narrow rifts in the bark. In 1917 the æciospores were not found infecting *Ribes* spp. at a greater distance than a hundred

yards; but the season was dry, and this factor may have limited the area of infection.

The uredinial stage (Pl. 57) of the rust on species of Ribes follows the infection by æciospores at the end of 12 to 15 days, or in cool weather a few days longer. In nature the reinfection of the species of Ribes by urediniospores may follow in about two weeks, the time being largely influenced by physical conditions, thus continuing the reinfection until the end of the growing season. It is not uncommon to find an abundance of uredinia on *R. aureum* and *R. odoratum*, with viable urediniospores in late autumn, even after freezing weather begins. Both the æciopores and unrediniospores undoubtedly are chiefly wind-disseminated. Insects may carry them to some extent. Cattle, sheep, and goats feed on *R. aureum*, brushing against the diseased leaves. During moist weather they are probably instrumental in spreading the disease.

The telial stage (Pl. 57) in nature ordinarily follows the uredinial stage in one to two weeks, varying somewhat with the temperature of the air and the age of the infected leaves, the controlling conditions not being well known. The mature teliospores germinate *in situ*, the telia assuming a silvery tinge, owing to the presence of sporidia. Pines in turn are infected by the sporidia, the pycnial and æcial stages following in succession after a period of one or probably more years, the time probably varying as in other species of Cronartium (*4*, *6*).

Cronartium occidentale seems to be able to overwinter on *Ribes aureum*. This is indicated by collections of the disease by one of the writers (Bethel) from the same clumps of *R. aureum*, and even from the same bushes, year after year in the parks of Denver and Boulder. The first collections of the disease at these places were made in 1909. It was subsequently found in the same places each year until 1917, when a severe drouth seems to have killed the organism in all of the clumps on which the disease had previously been found. The only known natural occurrence of the disease in either city during 1917 was a scattering infection found by Mr. Hunt in a group of plants of *R. aureum* in Washington Park, Denver. Conditions were such that it is quite possible the disease will not reappear there. It seems inconceivable that æciospores could be blown 50 miles or more and infect the same plant or groups of plants year after year, while many plantations near by remained free from infection.

Æcial infections usually occur on more or less scattered leaves all over the plants infected, and not on a single well-protected branch in the center of a clump. The greater part of the collections of the uredinial and telial stages of the fungus in 1917 were made in localities too remote from piñons for any probable æcial infection, and the manner of development indicated that the rust had in some way lived over the winter on the plants. In a few cases remote from piñons in 1917 the uredinial stage was observed at first on a single well-protected branch of a large

clump of *Ribes* sp. growing in a moderately damp place. Uredinia and telia are common on the petioles of the leaves and are occasionally found on the flower peduncles, but so far have not been observed on the stems. Posey, Gravatt, and Colley (5) have, in the case of *Cronartium ribicola*, found natural stem infections of *Grossularia hirtella*, which bore normal urediniospores. *Melampsora bigelowii* Thüm., also a common rust in Colorado, is known to overwinter on stems on *Salix* spp. here and elsewhere.

During a succession of seasons favorable for the spread and overwintering of *Cronartium occidentale* it might be distributed in the uredinial stage to regions far distant from piñon pines. *Coleosparium solidaginis* (Schw.) Thüm. on species of Aster and Solidago, which has for its æcial stage *Peridermium acicolum* Underw. and Earle, a needle-rust of pines, spreads across the plains for long distances in this way. *Coleosporium ribicola* (C. and E.) Arth. on species of Grossulariaceae is found in Montana nearly 400 miles to the north of the range of *Pinus edulis*, its only known natural æcial host. The occurrence of *Cronartium occidentale* at Stockton, Kans., in 1892, as reported by Arthur (2) may be explained similarly. Its occurence sporadically in the parks of Denver and Boulder may have been the result of large plantings with stock of *Ribes aureum* shipped from Colorado points where the disease is epidemic. Shipments are known to have been made from Rifle, where the disease was especially abundant in 1917.

In the spring of 1917 many infected leaves in a good state of preservation, bearing urediniosori which still had their natural color, were collected in the open at Denver and Bayfield, Colo., and used in inoculation experiments at Washington, D. C., without positive results. Attempts to germinate the urediniospores at the latter place failed also. The possibility that such urediniospores may remain viable over the winter should be investigated more thoroughly.

EFFECT OF THE FUNGUS ON ITS HOSTS

Peridermium occidentale is rarely found on very old trees of *Pinus edulis*, and in these it is in the crevices of the bark often with no adjacent dead areas. In such cases even when fruiting it is hardly discernible with a hand lens. Its effect on young trees is more apparent. A number of young trees apparently killed by the fungus were found in southern Colorado. Such trees are usually attacked on the trunk and branches near the ground. Some become spike-topped (Pl. 56). Lesions on old trees usually are found from 2 to 8 feet from the ground.

Plants of species of Ribes and Grossularia attacked by *Cronartium occidentale* apparently suffer but slight injury, owing to the fact that the season's growth is made chiefly before the infection becomes heavy. In severe early attacks partial defoliation may result, usually late in the growing season. Repeated attacks may stunt somewhat the growth of

the plants, but none have been found killed from the effects of the fungus. On leaves attacked by the fungus the uredinia and telia cause a characteristic spotting. The spots vary in color from a light yellow-green before frost occurs to a purplish brown afterwards. This spotting is most pronounced on leaves of *R. aureum* and *R. odoratum*.

SUMMARY

The species of Cronartium native on *Ribes* spp. and *Grossularia* spp. in Colorado and Arizona is described for the first time and named "*Cronartium occidentale*."

The æcial stage of this fungus is proved to be a form of Peridermium on the piñons, or nut pines, *Pinus edulis* and *P. monophylla*, and is now given the form-name "*Peridermium occidentale*."

Cronartium occidentale is widespread throughout the piñon region of Colorado, extending into Arizona. Further surveys will no doubt greatly extend the known range of the species.

The common native host for the telial stage of this fungus is *Ribes aureum*, although it occurs occasionally on *R. odoratum*, *R. inebrians*, and *Grossularia leptantha*.

Cronartium occidentale has been successfully inoculated on *Ribes americanum*, *R. aureum*, *R. coloradense*, *R. giraldi*, *R. malvaceum*, *R. nigrum*, *R. glandulosum*, *R. sanguineum*, *Ribes* sp., *Grossularia inermis*, *G. missouriensis*, and *G. reclinata* × *G. hirtella*.

Peridermium occidentale, so far as known, attacks ony the piñon pines. Inoculations have been made on 23 species of pines to ascertain whether it will attack other kinds of pines.

Cronartium occidentale differs essentially from *C. ribicola*, cause of the white-pine blister-rust, especially in the æcial stage. A synopsis of these variations is given.

Cronartium occidentale is apparently able to overwinter and maintain itself independent of the æcial stage. Only circumstantial evidence in support of this view has been obtained.

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PLATE 54

Specimens from the type collection of the æcial stage (*Peridermium occidentale*) of *Cronartium occidentale* on *Pinus edulis* from Bayfield, Colo. One twig has an æcial cavity exposed by turning back the overlying bark.

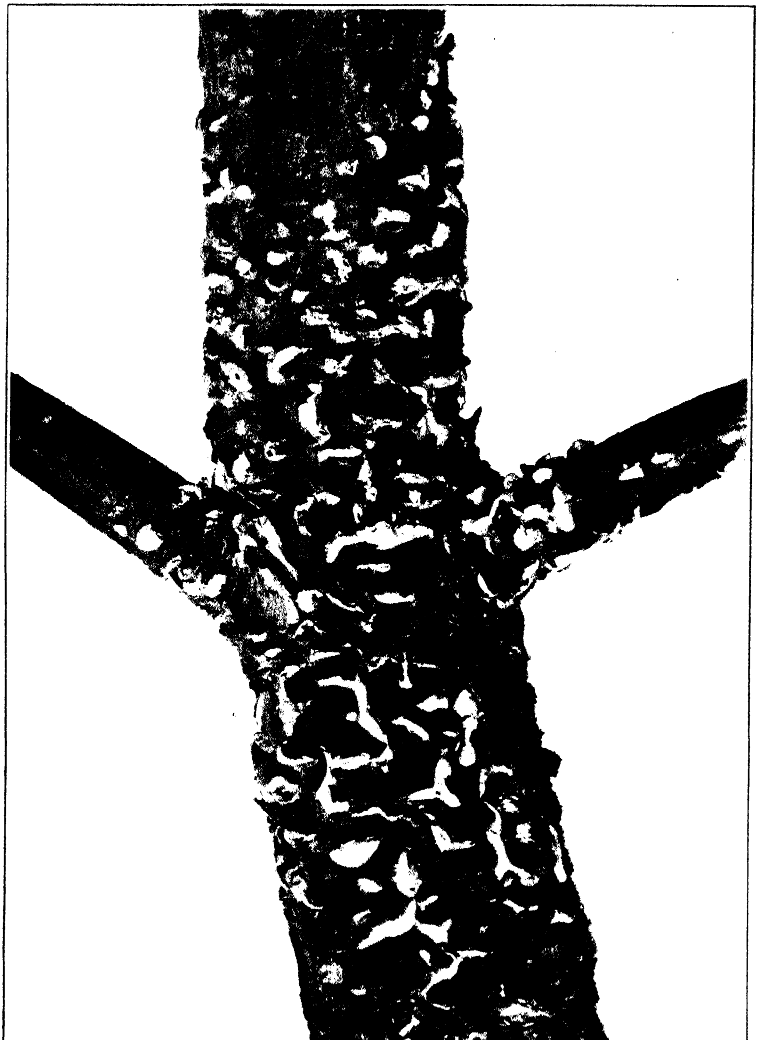

PLATE 55

The æcial stage (*Peridermium strobi*) of *Cronartium ribicola* on *Pinus strobus* from Kittery Point, Me. (Photographed by Dr. R. H. Colley).

PLATE 56

A young piñon tree (*Pinus edulis*) diseased with *Peridermium occidentale* in the pycnial stage, natural infection. Transferred from the forest near Mancos, Colo., to a pot in the greenhouse at Washington, D. C. The top has been killed by the fungus, giving it a spindle form, such as does not occur in trees fully alive.

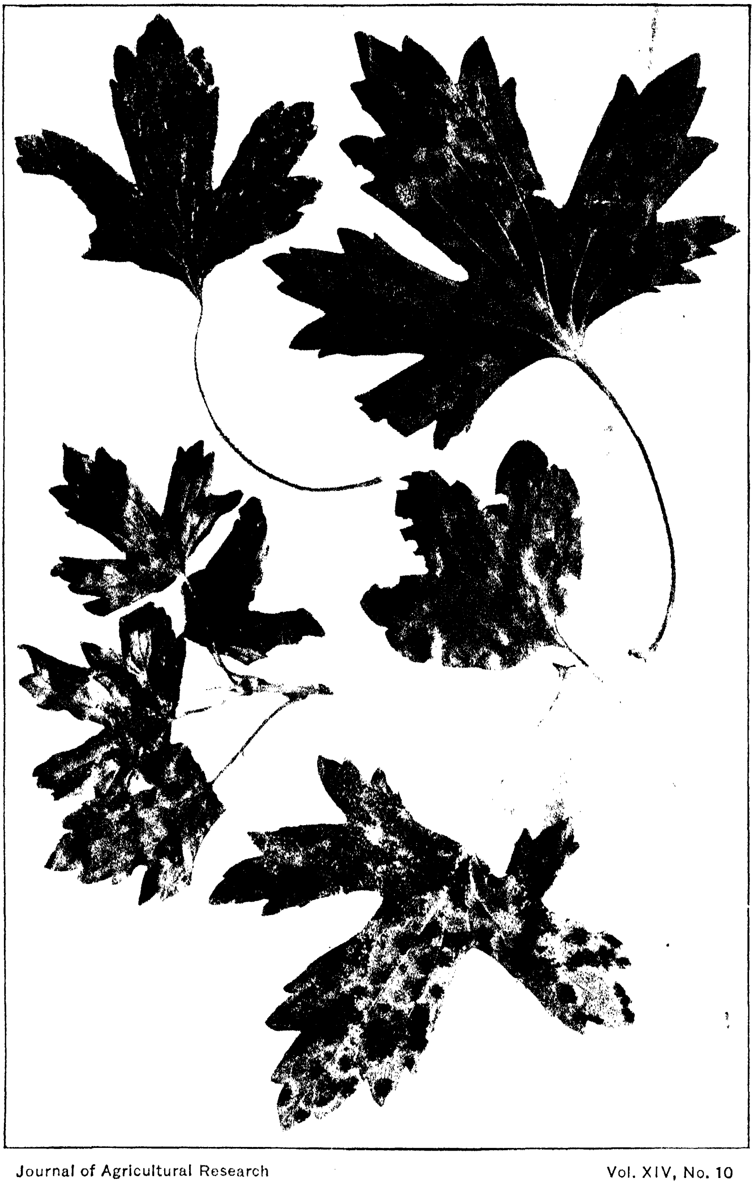

PLATE 57

The uredinial and telial stages of *Cronartium occidentale* on *Ribes aureum* from the type material collected at Bayfield, Colo.; artificial inoculation.

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COMPARATIVE TOXICITY OF COTTONSEED PRODUCTS

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INTRODUCTION

Notwithstanding the isolation by us of a distinctly toxic substance (gossypol) from cottonseed (*15*),[1] six papers have recently appeared offering to explain cottonseed-meal poisoning or "injury" on the theory of dietary deficiencies. The chief support for this view is found (a) by Rommel and Vedder (*13*) who fed pigs on polished rice and tankage developed symptoms similar to those fed at the same time on corn meal and cottonseed meal, and (b) by Richardson and Green (*10, 11, 12*) and by Osborne and Mendel (*7, 8*), who show that, while white rats will ultimately fail on diets containing cottonseed meal as the sole source of protein, minerals, and vitamines, the animals will grow normally on cottonseed-meal or cottonseed-flour diets if supplemented by milk powder, protein-free milk, butter, etc.

Wells and Ewing (*14*) have also adopted the dietary-deficiency view as being the most plausible explanation of cottonseed-meal injury. They base their conclusion that cottonseed meal is an incomplete food on experiments with very young pigs confined in metabolism cages, the cottonseed ration being supplemented with starch, sugar, and small quantities of milk. In some cases they admit that the injury may have been due to the presence of a toxic substance.

The fallacy in concluding, from the ultimate failure of rats on diets in which cottonseed meal is the sole source of protein, minerals, and vitamines, that dietary deficiencies are the cause of cottonseed-meal injury in swine, for example, is evident if we base a somewhat similar argument as to other seeds on the results of feeding them to rats as the sole source of these nutritive factors. For example, rats similarly restricted to any seed will show failure, and in most cases much sooner than on cottonseed meal, but we know that in the diet of swine these seed diets do not cause the sudden death which is typical of cottonseed-meal injury. Another distinct difference between the failure of rats on restricted cottonseed-meal diets and the failure of swine on cottonseed meal under farm conditions is that with rats death usually follows a period of low food intake and consequent emaciation, while with swine it is very well known that death often follows a period of excellent growth and with the animals in splendid nutrition. None of these investigators has fed

[1] Reference is made by number (italic) to "Literature cited," p. 451–452.

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diets designed to reveal whether the failure of their animals was due to inadequate diets or to the presence of a mildly toxic substance. We believe we have accomplished this by comparing ether-extracted cottonseed kernels with cottonseed meal.

After the appearance of Rommel and Vedder's (*13*) preliminary note, we conducted some preliminary experiments with pigs (*16*) in which we found gossypol isolated from raw cottonseed to be markedly toxic to these animals and also demonstrated that while animals on cottonseed meal quickly sickened and died, pigs which were fed on the ether-extracted cottonseed kernels were not affected. This fact seems to us to outweigh the hypothesis advanced regarding deficient diets as the cause of cottonseed-meal injury, and to confirm our previous view. With reference to the presence of a deleterious substance in cottonseed meal, we would emphasize at the outset the fact that pigs, rats, and rabbits grow better on a diet containing ether-extracted kernels than on a diet containing cottonseed meal. Pigs and rabbits are so quickly affected by cottonseed meal that we were led to state (*15*) that—

> generally speaking, the meal and the kernels are toxic to the same degree.

Many subsequent experiments by us with four species of animals have shown that meal is far less toxic than kernels for rats and hens, but the fact remains that cottonseed meal, even a thoroughly cooked meal, is highly injurious to rabbits and pigs.

Following the paper by Richardson and Green (*10*) we fed rats on cottonseed products. The vast difference between the highly toxic raw seed and the very slightly toxic cooked product was immediately evident. This indicated to us that in the manufacture of cottonseed meal the gossypol undergoes some change whereby the meal is rendered much less toxic than the original kernels.

In this paper we report some of the experiments conducted to ascertain to what extent the change in toxicity takes place under oil-mill conditions. These experiments led to the conclusion that there still remained a toxic factor in all the samples of cottonseed meal and cottonseed flour which we fed. Rats and hens are less affected by this factor than rabbits and swine. In fact, in diets well supplemented with milk powder the toxic factor for rats may remain entirely masked.

OIL-MILL TREATMENT OF COTTONSEED PRODUCTS

In order to compare the effects of cooking on the toxicity of cottonseed, we have used products obtained from cottonseed-oil mills. For comparing the effect on different species, two meals were extensively fed. These, together with other samples, were collected by one of us (Carruth) personally at the oil mills. Information was also obtained regarding the length of time, steam pressure, and other details of the cooking process. It is evident to us that the

"home-made" products of Osborne and Mendel do not resemble mill products. For example, their raw kernels "subjected to vigorous treatment with live steam from one to six hours," in which there was some loss through distillation and spraying, underwent a change in toxicity much more slowly than in the commercial process. In particular, their laboratory treatment of the kernels made the mass so wet that it had to be dried before feeding. This is not the condition in the oil mill, where air is often drawn through the cooking drum to remove excess moisture. Under mill conditions the kernels undergo a much more rapid reduction in toxicity, for we have found oil-mill samples after 25 to 28 minutes' cooking not markedly injurious to rats in adequate diets, while Osborne and Mendel's (*8*) product cooked for one hour was found by them to be appreciably toxic. After four hours' cooking under laboratory conditions their product became less suitable for nutrition, and the results led them to assume that undue heating might render the meal unpalatable. This also seems to us unlikely to happen in the better regulated conditions of moisture and heating in the industry.

Another point of marked difference is the fact that their actually cold-pressed oil was nontoxic, whereas we have found that the commercial so-called cold-pressed [1] oil is highly toxic because most of the gossypol from the resin glands has been dissolved by the oil and squeezed out unchanged.

Our experiments (*16*) with raw kernels and gossypol led us to believe that gossypol is the only toxic substance in the raw cottonseed. The toxicity of the cottonseed meal varies with the amount of unchanged gossypol present. But from certain meals which we found definitely injurious to rabbits and swine no gossypol could be isolated by our methods. Apparently it had been entirely changed in the cooking process to a very similar substance, called by us "D-gossypol," which is much less toxic but which can not be regarded as being without physiological action.

In the milling of cottonseed the decorticated kernels are cooked in steam-jacketed drums while being continually stirred with huge paddles. The mass becomes somewhat moist as the temperature rises, either from moisture present in the seed or from steam sprayed in, if the seeds are too dry. In this condition the kernels are quickly comminuted. These conditions are favorable for effecting an important change in the gossypol which issues from the glands and then spreads over the surface of the seed particles. The nature of

[1] The "cold-pressed" meal is made from kernels passed through preheaters surrounded by steam under pressure. This serves to drive out the moisture. The dried kernels are then ground in a screw press to express the oil. This also develops much heat from friction. The actual temperatures used in the hot pressing and cold pressing do not differ greatly. The latter is really a dry pressing rather than a moist pressing.

this change has not been ascertained, but it seems plausible to suppose it is an oxidation process.[1] The gossypol appears to be converted into D-gossypol, which is very similar in color reactions to gossypol, but which is much less soluble and has a higher melting point. In the hot-pressing process the highly toxic gossypol, under favorable conditions, is quickly changed to this considerablv less toxic substance.

COOKING PROCESS

The two meals which we used so extensively may be designated as the "short-cooked" meal and the "long-cooked" meal. The short-cooked meal was obtained from an oil mill using single "cookers," or steam-jacketed drums. The raw kernels after decortication and crushing are conveyed to the drum, where they are stirred by large revolving paddles. The steam pressure in the jackets was about 40 pounds. The kernels themselves are not subjected to steam pressure. In this drum the kernels are cooked for 28 minutes; then the drum is emptied; and the kernels are ready for the "cake former." In the meantime they are kept hot. The cake former allows a definite amount to pass out onto haircloth mats. The mat is folded and molded by light pressure and then carried to the hydraulic press. When enough cakes have been molded, pressure is exerted and maintained until the oil ceases to drip. The hard cake is then removed, usually allowed to cool, and then ground into the meal of commerce. From this particular mill sufficient meal was obtained for the experiments with rats, rabbits, fowls, and swine. In order to follow the change in toxicity on cooking, we took samples from the cooking drum at 5-, 10-, and 20-minute periods and also a sample of the kernels cooked the full period—28 minutes in this case. These products were all fed to rats as described on pages 431–431 (see fig. 1). A sample of oil from these fully cooked kernels was also obtained; however, as it contained practically no gossypol, it was not fed, but was assumed to be nontoxic, as was a similar sample secured from this mill at another time.

The long-cooked meal was obtained from a mill using a 5-"stack" (or drum) "French continuous cooker." In this type the steam-jacketed drums are placed one over another. The kernels enter the top drum and pass down as the drum below is emptied. In this mill the cooking operation consumed about two hours. The kernels and meal were considerably darker than the short-cooked meal and would be considered as off color, the meal being almost reddish brown instead of the usually highly regarded "bright-yellow" meal. Samples of fully cooked kernels and oil

[1] It is possible that this change may be otherwise effected in the unknown conditions of the colloidal material. Our reason for believing that it is not due to heat alone is that B-gossypol obtained by heating gossypol to 180° C. differs in properties from D-gossypol. This temperature is about 80° higher than the temperature of cooking cottonseed in the manufacture of cottonseed meal.

therefrom were also secured. The steam pressures in the jackets surrounding the various drums ranged from 20 to 40 pounds.

Since this long cooking period represents the upper limit of time and thoroughness of cooking in the cottonseed industry, the results of our rabbit and swine experiments especially are highly significant, in view of the claims that cottonseed-meal injury is a matter of dietary deficiency,

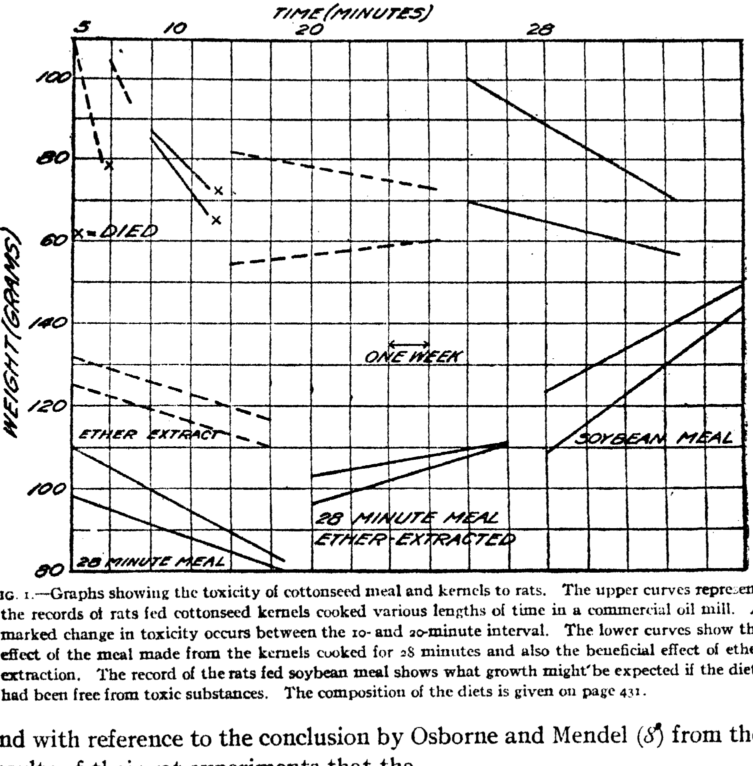

FIG. 1.—Graphs showing the toxicity of cottonseed meal and kernels to rats. The upper curves represent the records of rats fed cottonseed kernels cooked various lengths of time in a commercial oil mill. A marked change in toxicity occurs between the 10- and 20-minute interval. The lower curves show the effect of the meal made from the kernels cooked for 28 minutes and also the beneficial effect of ether extraction. The record of the rats fed soybean meal shows what growth might be expected if the diets had been free from toxic substances. The composition of the diets is given on page 431.

and with reference to the conclusion by Osborne and Mendel (8) from the results of their rat experiments that the—

treatment of cottonseed so as at least to render it harmless now seems to lie within the range of ready possibilities.

A sample of the long-cooked meal used in the pig diets, also in the rabbit, rat, and poultry diets, was sent to Dr. T. B. Osborne, at the Connecticut Agricultural Experiment Station, who wrote as follows:

Our results with the cottonseed meal from the French cooker show that this treatment has apparently entirely destroyed the toxic properties of the seed. All of our rats on this product are growing finely.

In these experiments Dr. Osborne supplemented the meal with protein-free milk and butter fat. It is evident that the results with rats do not indicate what effect meal has on rabbits and pigs.

EXPERIMENTAL WORK

EXPERIMENTS WITH RATS

On cottonseed meals which contain no unchanged gossypol, but apparently only the D-gossypol, rats have not done so well as on ether-extracted raw kernels which contain no D-gossypol and only traces of unextracted gossypol. (See Table I, diets 390, 392, 393, 383, and 383A; see also fig. 2, 3.) This fact leads us to maintain that there is still a toxic factor in well-cooked cottonseed meals. The difference holds true of the restricted cottonseed diets, which are practically identical with respect to protein, minerals, and vitamines. When these products are supplemented with 17 per cent of milk powder, there is no

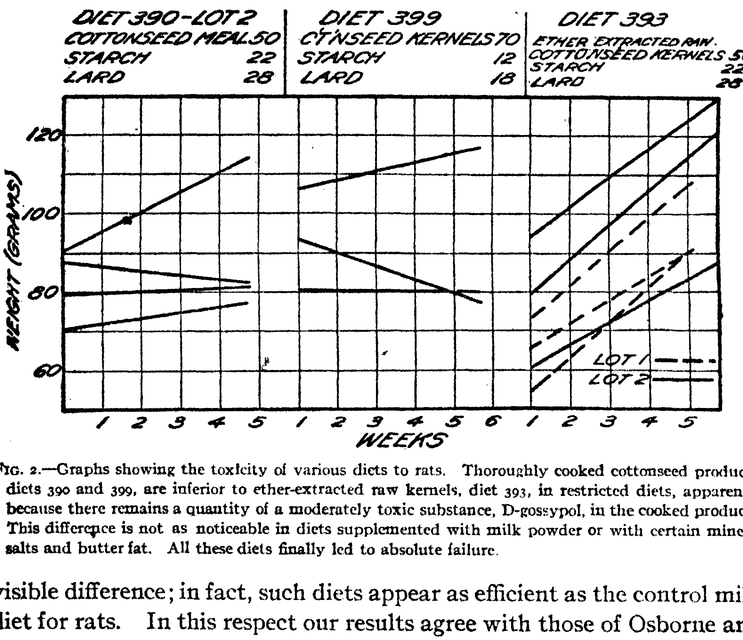

FIG. 2.—Graphs showing the toxicity of various diets to rats. Thoroughly cooked cottonseed products, diets 390 and 399, are inferior to ether-extracted raw kernels, diet 393, in restricted diets, apparently because there remains a quantity of a moderately toxic substance, D-gossypol, in the cooked products. This difference is not as noticeable in diets supplemented with milk powder or with certain mineral salts and butter fat. All these diets finally led to absolute failure.

visible difference; in fact, such diets appear as efficient as the control milk diet for rats. In this respect our results agree with those of Osborne and Mendel (*8*) and Richardson and Green (*10, 11, 12*).

The rats used were bought from a dealer and were apparently inferior animals both in size and vitality. Few of them exceeded 200 gm. in weight, and many ceased growing at 100 to 125 gm. In the experiments animals from the same shipment were used in one set so that the results are generally but not always comparable with the results of other sets. In only one case out of many experiments with rats on unsupplemented cottonseed meal (long-cooked) diets have our rats shown appreciable growth. This instance (feed 390, lot 5) was with two rats from a litter reared in our animal rooms. The mother and young were fed on the control milk diet with occasional green food until

the young were 55 days old. During the subsequent 31 days these two rats made excellent gains; in fact, they grew nearly as well as the rest of the litter on the milk diet. It is quite possible that the previous diet may explain why they made such a better record. It is well-known that rats supplied by dealers are often less vigorous, probably owing to the fact that often they have been reared on insufficient diets.

TABLE I.—*Effect of various diets on the growth of rats*

UNSUPPLEMENTED DIETS

Experiment and diet No.	Number of rats.	Diet.	Average weight. Initial.	Average weight. Final.	Change.	Duration of experiment.
EXPERIMENT 1:			*Gm.*	*Gm.*	*P. ct.*	
390, lot 1	3	Cottonseed meal, 50 per cent.	52	.....	.....	Died in 9, 17, and 47 days.
392	3	Cottonseed flour, 50 per cent.	50	.....	.....	Died in 9, 16, and 47 days.
393, lot 1	3	Ether-extracted cottonseed kernels, 50 per cent.	65	99	+52	Discontinued after 29 days.
385	3	Soybean meal, 50 per cent; crude cottonseed oil, 14 per cent.	70	85	+21	Discontinued after 22 days.
EXPERIMENT 2:						
399	3	Cottonseed kernels cooked 2 hours, 70 per cent.	94	91	− 3	Discontinued after 33 days.
390, lot 2	4	Cottonseed meal from above kernels, 50 per cent.	82	89	+ 8	Do.
393, lot 2	3	Ether-extracted cottonseed kernels, 50 per cent.	78	112	+44	Do.
EXPERIMENT 3:						
390, lot 5 (see p. 430).	2	Cottonseed meal as in experiment 2, 50 per cent.	101	143	+42	Discontinued after 31 days.
Do	2	Milk diet (control).	89	136	+53	Do.

SUPPLEMENTED DIETS [a]

Experiment and diet No.	Number of rats.	Diet.	Initial.	Final.	Change.	Duration of experiment.
EXPERIMENT 4:						
424	1	Cottonseed kernels cooked 5 minutes.	109	78	−28	Died in 5 days.
425	2	Cottonseed kernels cooked 10 minutes.	86	71	−18	Died in 9 days.
426	2	Cottonseed kernels cooked 20 minutes.	68	68	− 0	Discontinued after 37 days.
427	2	Cottonseed kernels cooked 28 minutes.	85	65	−23	Do.

[a] Diets 424 to 427, inclusive, contained 70 per cent of cottonseed kernels, 12 per cent of starch, and 18 per cent of lard. Diets 428 to 433 contained 50 per cent of meal, 22 per cent of starch, and 28 per cent of lard. The cottonseed kernels used in diets 424, 425, 426, and 427 contained, respectively 0.62, 0.24, 0.10, and 0.07 per cent of unchanged gossypol.

TABLE I—*Effects of various diets on the growth of rats*—Continued

UNSUPPLEMENTED DIETS—continued

Experiment and diet No.	Number of rats.	Diet.	Average weight. Initial.	Average weight. Final.	Change.	Duration of experiment.[a]
EXPERIMENT 4—Continued.			*Gm.*	*Gm.*	*P. ct.*	
428	2	Cottonseed meal made from kernels cooked 28 minutes.	104	80	−23	Do.
429	2	"Buco" cottonseed meal cooked (?) minutes.	66	73	+11	Discontinued after 38 days.
432	2	28-minute cottonseed meal, ether-extracted.	99	111	+12	Discontinued after 35 days.
431	2	Ether extract of 28-minute cottonseed meal, 12 per cent.	129	114	−12	Do.
433	2	Soybean meal	116	147	+27	Do.
435	6	28-minute cottonseed meal, 45 per cent; whole milk powder, 17 per cent.	71	114	+61	Discontinued after 29 days.
EXPERIMENT 5:						
372	3	Ether extract of cottonseed flour, 28 per cent.	82	76	− 7	Discontinued after 10 days.
372A		Ether extract of cottonseed flour, 7 per cent.	76	106	+39	Discontinued after 21 days.
372B		Ether extract of cottonseed flour, 10 per cent.	106	124	+17	Do.
388	2	Petroleum-ether extract of cottonseed kernels, 14 per cent.	199	208	+ 4	Discontinued after 40 days.
388A		Kernels extracted by petroleum ether.	130	119	− 8	Discontinued after 3 days. Refused to eat.
440	2	Crude cold-pressed cottonseed oil, 14 per cent (gossypol 0.21 per cent).	115	81	−30	Discontinued after 14 days. Fatal to one.
438	2	Cold-pressed cake meal (mill M), 40 per cent with milk powder.	105	111	+ 6	Discontinued after 10 days.
438A		Cold-pressed cake meal (mill M), 50 per cent, with starch and lard only.	110	93	−15	Do.
439	2	Cold-pressed meal from mill E, 50 per cent.	110	108	− 2	Discontinued after 8 days.

[a] The rats on diet 435 were the emaciated animals previously on diets 426, 427, and 428.

TABLE I—*Effects of various diets on the growth of rats*—Continued

UNSUPPLEMENTED DIETS—continued

Experiment and diet No.	Number of rats.	Diet.	Average weight. Initial.	Average weight. Final.	Change.	Duration of experiment.
EXPERIMENT 5—Continued.			*Gm.*	*Gm.*	*P. ct.*	
451	2	Cold-pressed meal from mill W, 50 per cent.	148	152	+ 3	Discontinued after 15 days.
[a] 460	2	Hot pressed meal from mill T, 50 per cent.	126	109	−13	Discontinued after 14 days.
460A	3	Ether extract of above 12.5 per cent, with milk powder.	143	116	−17	Discontinued after 10 days.
EXPERIMENT 6:						
380	3	Soybean meal, 70 per cent; lard, 30 per cent.	45	51	+13	Discontinued after 79 days. Failure.
381	3	Soybean meal, 25 per cent; corn meal, 75 per cent.	46	47	−2	Discontinued after 30-40 days. Failure.
375	3	Cottonseed meal, 25 per cent; corn meal, 75 per cent.	46	37	−20	Discontinued after 50 days. Failure.
383	3	Period 1: Cottonseed flour, 30 per cent; corn meal, 42 per cent; lard, 28 per cent.	67	79	+18	Discontinued after 68 days. Stopped growing entirely.
383A		Period 2: Ether-extracted cottonseed kernels in place of cottonseed flour (fig. 3.)	79	106	+34	Discontinued after 68 days; better growth than during period 1.

[a] The meal in diet 460 contained some unchanged gossypol.

In experiment 1 the rats on the extracted kernels made fair progress until failure set in after 140 days. Rats on soybean meal failed after 128 days. These diets contained 50 per cent of the food under experiment, together with 22 per cent of starch and 20 per cent of lard.

In attempts to find a cottonseed meal which would be markedly toxic to rats, even in milk-powder diets, we fed several samples of so-called cold-pressed meal. Here, also, we failed to find marked toxicity. This was puzzling at the time, but investigation showed that in the so-called cold-pressing a large amount of the gossypol passes into the crude oil, which is highly toxic, and the remainder is more or less changed, so that there is nothing inconsistent in these results. It may be mentioned that by actually using cold pressure on raw cottonseed kernels, Osborne and Mendel obtained an oil which was not appreciably toxic, though the

resultant meal was highly toxic. We have shown practically the same thing by feeding kernels and oil extracted by petroleum ether (table I, diets 388 and 388A).

It would seem that by the extraction with ethyl ether of certain cottonseed meals and flour, a small amount of toxic substance is removed. Thus, 28 per cent of the oil extracted from the flour was unpalatable to rats in a milk-powder diet; but when reduced to 7 and 10 per cent, it failed to have any noticeable influence (compare diets 372, A and B, Table I). In diets 428, 431, and 432 the ether-extracted meal was less active (fig. 1).

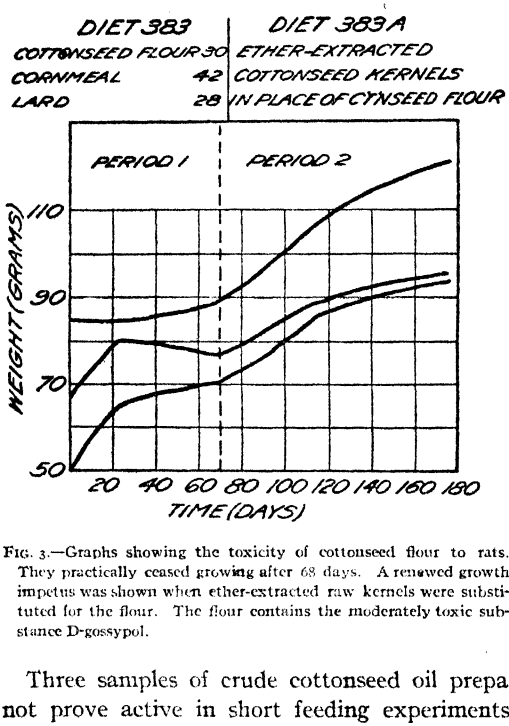

FIG. 3.—Graphs showing the toxicity of cottonseed flour to rats. They practically ceased growing after 68 days. A renewed growth impetus was shown when ether-extracted raw kernels were substituted for the flour. The flour contains the moderately toxic substance D-gossypol.

It should be noted here that cottonseed meal is not rendered nontoxic for rabbits (see diet 411, Table II) by ethyl-ether extraction, a fact which seems in harmony with the fact that in the extracted meal there is left a substance which responds to the tests for gossypol. The incomplete removal of this material thus lessens the decisive value of experiments with extracts from cottonseed meal.

Three samples of crude cottonseed oil prepared by hot pressing did not prove active in short feeding experiments. Tests made previous to feeding showed only traces of gossypol, and hence led us to predict this result. On the other hand, we found that in the commercial cold-pressing process most of the gossypol passed into the crude oil. This oil, which contains at least 1.5 per cent of gossypol, was found to be highly toxic (see diet 440, Table I, commercial cold pressed). The meal was not more active than short-cooked cottonseed meal (compare diets 439 and 451, Table I.)

Except in rare instances, cottonseed meal is fed in combination with other feeds. It has been found at this Station that steers fed exclusively on cottonseed meal and hulls do not thrive as well as where the roughage is silage. The meal and hulls in combination may not be fed safely for more than 70 to 90 days.

With pigs from 10 to 50 per cent of cottonseed meal has been fed. The usual experimental diet at this Station has been cottonseed meal 25 parts, corn or corn meal 75 parts. This diet soon causes pigs to diminish their food intake, and serious results will ensue if the pigs are kept on this ration. Deaths occur frequently after five weeks. Undoubtedly, even with no toxic factor present, a severely restricted diet of this type, as corn with other protein supplements, such as soybean meal, peanut meal, etc., is inadequate for normal growth and reproduction of pigs. It would appear from the insufficiency of a vegetarian diet that rats would also fail to make normal growth in diets of this type; compare Slonaker (*6*) and McCollum et al. (*5*, *6*).

A few experiments with rats have confirmed this opinion, and from the improvement of animals when given salts and butter fat it would appear that rations of this type contain an unsuitable inorganic basis and insufficient amounts of the fat-soluble A. This seems to be in harmony with our knowledge of the common seeds which have been studied by McCollum and his associates.

Thus, young rats on cottonseed meal and corn meal (1:3) failed to grow and ultimately died, but not sooner than rats on soybean meal and corn meal fed in the same proportion. Some older rats maintained their weight for 40 days, but also finally failed.

The addition of calcium, sodium, and chlorin, elements in which the ash is very poor, and the addition of butter proved beneficial after some of the survivors had begun to decline.

These experiments with soybean meal were conducted to aid in the interpretation of the cottonseed experiments. The percentage of protein and the inorganic content of soybean meal are very similar to that of cottonseed meal.

Experiments (unpublished) with pigs by the Animal Industry Division of this Station have shown that soybean meal is vastly superior to cottonseed meal for pigs; still, when the animals are confined to pens and restricted to corn and soybean meal, the appetite is impaired after several months, and a sort of pathological lameness and stiffness of the legs appears.

The facts stated above show that these protein concentrates in restricted diets may ultimately cause failure. This phenomenon is, in our experience, entirely distinct from the sudden death of pigs on cottonseed meal, which may follow excellent growth.

EXPERIMENTS WITH RABBITS

Rabbits have been used extensively for experimental feeding of cottonseed products at this Station. The general methods of feeding were described in our previous article (*16*). Under our conditions young rabbits grew rapidly and grown rabbits fattened or maintained weight on

control diets of corn meal or soybean meal and molasses instead of cottonseed meal. On cottonseed products the rabbits showed varying behavior, from being made sick (diarrhea) on one or two doses of raw kernels to consuming the ether-extracted kernels without noticeable harm for 75 to 200 days. Toward various cottonseed meals the rabbits also showed varying reactions. Some meals not thoroughly cooked were refused in less than a week, while other meals were eaten for more than three or four weeks before the rabbits became sick. The same general results were observed in these as in the rat experiments. The ether-extracted raw kernels were least toxic of all, but the rabbits were rather sensitive even to them, and occasionally became sick after long feeding periods when fed at the rate of 1 per cent of body weight daily, the usual rate of feeding. This is apparently due to incomplete extraction of the kernels (Table II).

TABLE II.—*Effect of various diets on the growth of rabbits*

Diet No.	Number of rabbits.	Food.	Average weight. Initial.	Average weight. Final.	Change.	Percentage food eaten is of initial weight.	Duration and result.
			Gm.	*Gm.*	*Gm.*		
325	6	Raw cottonseed kernels.[a]	1,620	1,477	−143	2.7	Discontinued after 6–26 days. Refused to eat much. Made sick after 10–20 gm. were eaten.
349	4	.....do.............	2,332	2,000	−332	3.8	Discontinued after 15–42 days. Refused to eat.
350	7	Ether-extracted cottonseed kernels.[a-b]	1,620	2,025	+405	75.0	Discontinued after 73–204 days. Occasionally went off-feed.
...	[c]3	Cottonseed flour.....	455	527	+ 72	9	All died in 12 days.
350	[c]3	Ether-extracted cottonseed kernels.	392	527	+135	20	Discontinued after 16 days. Not affected.
407	3	Soybean meal.......	1,015	1,515	+500	69	Discontinued after 57 days. In normal health.
406	3	Long-cooked cottonseed meal, No. 1.	1,038	1,033	− 5	26	Died in 21–40 days.
410	3	Short-cooked cottonseed meal.	1,278	1,164	−114	17	All off feed in 13 days; 2 died (22d and 24th days); 1 refused to eat on 31st day.
411	3	Short-cooked, ether-extracted cottonseed meal.	733	945	+212	35	All died in 24–35 days.
408	3	Long-cooked cottonseed meal, No. 2.	1,133	1,197	+ 64	35	All sick in 35 days; 1 died on 38th day.

[a] Molasses fed with morning feed; collards and fresh greens for roughage.
[b] Average daily intake of food per rabbit was 10.6 gm.
[c] Very young rabbits from same litter.

TABLE II.—*Effects of various diets on the growth of rabbits*—Continued

Diet No.	Number of rabbits.	Food.	Average weight. Initial.	Final.	Change.	Percentage food eaten is of initial weight.	Duration and result.
			Gm.	*Gm.*	*Gm.*		
409	3	"Buco" cottonseed feed.	1, 515	1, 648	+133	35	Discontinued after 37 days; 2 died in 40 and 44 days; 1 not affected and bore 5 young.
433	2	Long-cooked cottonseed meal, No. 3.[a]	1, 333	1, 175	−158	......	Off feed in one week; died in 26 days.
...	3	Long-cooked cottonseed meal, No. 2.[b]	1, 637	1, 507	−130	19	One died on the 19th day, 1 on the 22d day, 1 slightly off-feed in 27 days.
...	3	Ether-extracted cottonseed kernels.[b]	1, 707	1, 868	+161	27	Discontinued after 27 days. In good health.

[a] With dry alfalfa for roughage. [b] With fresh vetch for roughage.

With such a range of gradations and with the animals being distinctly affected in three to four weeks by even thoroughly cooked meals the results do not appear to us to be due to any lack of dietary essentials, especially in view of the very liberal feeding of fresh green food daily (collards, pea vines, vetch, clover, etc.).

Although there was never the slightest evidence that the molasses mixed with the cottonseed products for feeding had any bad effect, it was thought advisable to feed the meal in a different type of diet. Accordingly the animals were restricted to a monotonous dry diet containinf 40 per cent of alfalfa meal for roughage, 20 per cent of corn meal, 10 per cent of ground oats, and 30 per cent of the cottonseed product or protein concentrate to be compared (Table III). It was found that the same relations held true of cottonseed meal, except that the animals were thrown off their feed considerably quicker than on the regular experimental diet. The rabbits were not made sick by the ether-extracted kernels nor by soybean meal, but were quickly affected by the cottonseed-meal diets. The soybean meal was fed after the animals had been made sick on cottonseed-meal diets, and on this diet they regained normal appetite and weight. The long-cooked meal and the short-cooked meal were the same products as used in the rat, hen, and pig diets.

The great sensitiveness of the rabbit to cottonseed meal may be made clear by comparing the upper limit of toleration of the meal with the weight of meal which hens of similar weight withstood without being seriously affected. On various meals the rabbits sickened or died after

eating 17 to 35 per cent of their initial body weight when fed at the rate of 1 per cent or less per day, while hens withstood 600 to 700 per cent when fed at the rate of 1.25 to 1.6 per cent of body weight daily.

TABLE III.—*Effect of monotonous dry diets containing 30 per cent of concentrates on the growth of rabbits*

Period and rabbits No.	Feed.	Average weight.		Change.	Duration and results.
		Initial.	Final.		
PERIOD 1:		*Gm.*	*Gm.*	*Gm.*	
14, 16	Short-cooked cottonseed meal.	1,408	1,180	−228	Discontinued after 9 days. Both ate little at end. No. 14 became sick and died on 15th day.
9, 10, 11	do	1,289	995	−284	Died in 16, 19, and 39 days. No. 9 gained at first.
29, 27	Long-cooked cottonseed meal 3.	1,254	1,095	−159	Discontinued after 15 days. Off feed.
13, 23	Long-cooked cottonseed meal 2.	1,873	1,505	−368	Do.
37, 38	do	978	1,038	− 50	Discontinued after 16 days. One died, the other improved on this diet plus ferric ammonium citrate.
39, 40, 41	Ether-extracted cottonseed kernels.[a]	982	1,337	+355	Discontinued after 23 days. In good health.
PERIOD 2:[b]					
16, 13, 23, 27, 29	Soybean meal	1,298	1,599	+301	Discontinued after 19 days. All in good health.

[a] After 1 month on this diet the rabbits seemed rather tired of it, but had continued to gain and showed no sign of sickness.
[b] Period 2: the rabbits which were off feed on the cottonseed mixtures were changed to a similar mixture containing soybean meal.

EXPERIMENTS WITH POULTRY

In order to ascertain whether excessive amounts of cottonseed meal had any pronounced effect on fowls and also to study the reputed effect of the meal on the pigmentation of the yolk, several short experiments were conducted. The fact that the birds were alive and laying to some extent at the end of 170 days indicates the slight extent to which hens are affected by cottonseed meal. The meal used was made from kernels cooked for two hours under the same conditions those used in the long-cooked meals of the rat, rabbit, and pig experiments. Later, in one diet a short-cooked (28 minutes) meal was used. The composition of the diets is given in Table IV.

TABLE IV.—*Percentage composition of the poultry diets*

Feed.	Lot 1 (5 hens).	Lot 2 (5 hens).	Lot 3 (5 hens).	Lot 4 (5 hens).	Lot 5 (5 hens).	Lot 6 (6 hens).
Gossypol	....	....	....	....	0. 2	....
Raw cottonseed kernels	....	....	....	....	....	30
Ether-extracted cottonseed kernels	....	....	....	40	....	....
Cottonseed meal, cooked two hours	40	30–0	30	....	....	....
Cottonseed meal, cooked 28 minutes	....	0–30	....	....	....	....
Meat meal	....	10	....	....	20–0	10
Corn meal	40–50	30	30	40–50	20–30	20
Wheat bran	....	10	20	....	30	10
Ground oats	....	10	20	....	19. 8	20
Whole milk powder	20–10	....	....	20–10	10. 20	10
Skim-milk powder	....	10	....	....	....	....
Protein (N × 6. 25)	23. 3	26. 3	18. 0	29. 0	28. 0	26. 0
Protein from cottonseed	13. 3	10. 0	10. 0	18. 8	0. 0	9. 3
Cracked limestone	Ad libitum.					
Tap water	Ad libitum.					

The meat meal (80–85 per cent crude protein) was discontinued in lot 5 after 32 days, as the birds had declined greatly. Additional milk powder (10 per cent) and additional corn meal (10 per cent) were substituted in an attempt to make the diet more appetizing. This reduced the protein content to about 17 per cent.

Lot 6 was started after all in lot 5 had died.

Owing to inability to secure enough milk powder, after 140 days the amounts used in lots 1 and 4 were reduced to 10 per cent. Later, it was necessary to make use of skim-milk powder plus butter.

After the pig, rat, and rabbit experiments had shown considerable difference in toxicity between the long-cooked and short-cooked meals, the hens in lot 2 were fed on the short-cooked meal from the eighty-fifth day in place of the long-cooked meal.

The protein content of 40 per cent of extracted kernels is equal to about that of 55 per cent of cottonseed meal used in lots 1, 2, and 3.

The fowls were fed under ordinary conditions by an experienced poultryman. Each lot was confined in outdoor turf-covered pens about 6 by 30 feet. Covered sheds opening to the south were provided for roosting and shelter. The fowls were gradually worked up to the maximum amount of feed given each lot (0.9 pound) daily. Weighings were made every tenth day. The dead fowls were examined by Dr. B. F. Kaupp, Poultry Expert and Pathologist of this Station, under whose supervision the experiment was conducted. The results of this experiment are given in Table V.

TABLE V.—*Effect of cottonseed products on the growth of hens*

Lot No.	Feed.	Number of birds.	Average age.	Average weight. Initial.	Average weight. Final.	Loss.	Period survivors fed.	Deaths and day of occurrence.
			Months.	*Pounds.*	*Pounds.*	*Per ct.*	*Days.*	
1	Long-cooked cottonseed meal, 40 per cent.	5	23	4.5	4.2	6.7	170	
2	Long-cooked cottonseed meal; short-cooked meal, 30 per cent.	5	25	4.4	4.2	4.5	170	
3	Long-cooked cottonseed meal, 30 per cent; no animal food.	5	24	3.9	3.0	23.1	170	2. 44th and 136th day.
4	Ether-extracted cottonseed kernels, 40 per cent.	5	31	4.7	3.8	19.1	170	2. 120th and 163d day.
5	Gossypol, 0.2 per cent.	5	17	4.3	2.8	35.0	......	5. 61st, 62d, 65th, 86th 91st day.
6	Uncooked cottonseed kernels, 30 per cent.	6	(?)	4.5	3.25	27.7	70	3. 28th, 55th, 70th day.

Lot 5 was fed in December, January, and February. Lot 6 was fed from March 10, when there was considerable grass, etc., in the yards. This enabled the animals to obtain food aside from the cottonseed mixture, and possibly explains why deaths were not so frequent as during a similar period in lot 5.

GENERAL EFFECT ON HEALTH OF HENS

Dr. Kaupp comments on the lots at the termination of the experiment as follows:

> Lots 5 and 6 suffered with pendulous crops, became sick of the feed, and all of lot 5 died. Lot 6 was following a similar route when discontinued. The birds in this lot were down in vitality.
>
> Lot 1 was in good physical condition except hen 8, which was suffering from pendulous crop. All the birds in lots 2 and 3 were in good physical condition. The birds of lot 4 suffered from pendulous crops, hen 19 being the worst. All the birds affected fully recovered, and were in good physical condition after a few days' feeding of normal rations for fowls.

There seems to be variance of opinion as to whether cottonseed meal is injurious to fowls. We may suggest that this is due to the use of different cottonseed meals. Thus, Hartwell and Lichtenthaeler (*3*), Clayton (*1*), and Osborne and Mendel (*7*) found no sign of toxicity. Kaupp (*4*), of this Station, has reported a high death rate among birds receiving large amounts of cottonseed meal, compared with control fowls fed on similar diets containing linseed meal.

The prompt effect of the diets of lots 5 and 6 on the health of the fowls indicates the highly toxic character of raw cottonseed and the gossypol therefrom. The effects of the other diet appeared after so long a period that possibly other factors may have been the cause of death in lots 3 and 4. But it will be noted that diet 3 was the poorest with respect to supplemental feeds of animal origin. Lot 4 received 40 per cent of ether-extracted cottonseed kernels, which is equivalent to about 55 per cent cottonseed meal, on the basis of the nitrogen content. This diet was therefore higher both in total protein and cottonseed protein. However, the fact that even this material has been found to be slightly toxic to rabbits and pigs would indicate that a toxic factor came into play. It was singular, however, that this lot was the only lot in which eggs were laid in the first part of the experiment. (See Table VI.)

To judge the results of the experiment by the effect on egg laying, the diet containing the ether-extracted cottonseed kernels, lot 4, was the least injurious of all during the first three months, while this relation was reversed with respect to cottonseed-meal lots during the last three months.

TABLE VI.—*Egg record (by months) of hens fed cottonseed products*

Lot No.	Food compared.	Dec.	Jan.	Feb.	Mar.	Apr.	May.	Total.
1	40 per cent cottonseed meal	0	0	0	32	31	7	70
2	30 per cent cottonseed meal	3	3	0	8	34	11	59
3	do	0	0	0	6	25	0	31
4	40 per cent extracted cottonseed kernels	13	17	29	0	2	0	61
5	0.2 per cent gossypol	0	0	0				0
6	30 per cent raw cottonseed kernels				1	0	10	11

Another noteworthy feature, indicating the high resistance of fowls, was the fact that the short-cooked meal, which was found distinctly more injurious to rabbits and swine, had no more effect on the fowls than the long-cooked meal.

AMOUNT OF FEED EATEN BY EACH LOT

The birds in these experiments were accustomed gradually to the diet. The amounts fed were gradually increased to a maximum of 0.9 pound (408 gm.) per lot per day of feed 81.7 gm. per bird containing from 24 to 32 gm. of cottonseed product. The diets of lots 5 and 6 were but poorly consumed, and there was consequently a steady loss in weight. The other feeds were eaten regularly, although at the end the birds in lot 4 were losing appetite. The record of the feed eaten is given in Table VII.

TABLE VII.—*Feed consumption of the six lots of hens fed cottonseed products*

Lot No.	Food compared.	Total feed consumed.		
		Per lot.	Per bird.	Cottonseed product.
		Pounds.	*Pounds.*	*Pounds.*
1	40 per cent cottonseed meal	148.7	29.7	11.9
2	30 per cent cottonseed meal	152.5	30.5	9.2
3	.....do	147.7	29.5	8.9
4	40 per cent extracted cottonseed kernels	132.6	26.1	[a] 10.6
5	0.2 per cent gossypol	29.5	5.8	[b] .0117
6	30 per cent raw cottonseed kernels	28.6	4.8	1.43

[a] This is equivalent to 14.6 pounds of cottonseed meal on basis of nitrogen content.
[b] This amount of gossypol (5.3 gm.) is equivalent to 1.75 pounds of raw cottonseed kernels.

EFFECT ON COLORATION OF THE YOLK

It has been noted frequently that eggs from hens fed cottonseed meal show a peculiar brownish discoloration of the yolk, giving them the appearance of very old eggs. Several eggs from each of the cottonseed-meal lots were examined, but seemed to be of normal appearance. There were no eggs laid by lot 5 (fed gossypol). Possibly, if a diet of intermediate toxicity between the diet of lot 5 and the diets of lots 1, 2, 3, and 4 had been fed—that is, so that laying was not entirely prevented—there would have been some abnormal appearance in the eggs. Out of 10 eggs examined in the lot fed raw cottonseed kernels the yolks of 4 were very perceptibly affected with the brown discoloration. The other yolks were affected but slightly, or not at all. The data are rather meager, but at least indicate that, when this phenomenon occurs in eggs, it may be due to gossypol which is unchanged, owing to insufficient cooking of the cottonseed. Whether this phenomenon is due to the peculiar pigments of the cottonseed or to some physiological action of gossypol on the fowl has not been ascertained. The authors are of the opinion that the discoloration is not due to the deposition of gossypol or related substance in the yolk, such as occurs with carotin and xanthophyll. This opinion is based on the fact that, although gossypol is a fat-soluble pigment, it readily forms salts which are not fat-soluble and is also so easily oxidized that one would not expect it to be stored as such in egg fat. Thus, it does not occur in milk fat or body fat of animals fed with cottonseed meal. In fact, Palmer and Eccles (*9*) have shown that cottonseed meal tends to produce a colorless butter, owing to the very small amounts of carotin and xanthophyll present.

EXPERIMENTS WITH SWINE

As a result of our preliminary experiments (*16*) with rats, rabbits, and pigs, in which it was shown that raw cottonseed and gossypol were highly toxic, it appeared that the cooked kernels, and therefore cottonseed meal, were decidedly less toxic by reason of some transformation of

gossypol. At the same time it appeared that cottonseed meal has nutritive limitations to which others have attributed the phenomenon of cottonseed-meal "injury." Such limitations are, of course, not peculiar to cottonseed, but exist in all seeds. Thus, corn, wheat, rice, oats, etc., have been shown by McCollum and associates to possess an insufficient supply of the fat-soluble growth-promoting substance. As a single source of protein, minerals, and vitamines for rats cottonseed meal seems far superior to any of these grains. In fact, cottonseed meal has an ash content about seven times greater than that of corn.

Hart, Miller, and McCollum (*2*) have shown that the wheat embryo contains a toxic material which is manifest in certain limited diets, but the effect of which is overcome in a highly efficient diet.

Recognizing the profound influence that cooking cottonseed meal has on the toxicity of the raw seed, we chose for our experiments two meals the preparation of which has been previously described in this article.

The objects of the pig experiments were to ascertain—

(1) Whether "injury" similar to cottonseed-meal "injury" was manifested in diets containing high protein concentrates whose nutritive properties we might expect to be similar to cottonseed meal.

(2) Whether a meal which had been subjected to cooking for a period which represents the upper limit in cottonseed milling would prove definitely injurious to swine.

(3) Whether, as we predicted, a short-cooked meal would prove more active than a long-cooked meal, other things being equal.

(4) Whether by improvement of the diet (diet 6) by the addition of of butter fat, meat scrap, and the inorganic elements (calcium, sodium, chlorin) which are the least abundant of the necessary elements in the meal, or by addition of milk products (diet 7), which would improve the protein, mineral, and vitamine content of the diet, cottonseed meal poisoning might be averted.

TABLE VIII.—*Percentage composition of the swine diets*

Feed.	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 5.	Lot 6.	Lot 7.
Cottonseed meal 1, long-cooked (2 hours)	25	.....	.....	.....	.....	30	30
Cottonseed meal 2, short-cooked (28 minutes)	.....	25	.....	.....	.....	.....	.....
Soybean meal	.....	.....	25	.....	.....	.....	.....
Peanut meal	.....	.....	.....	25	.....	.....	.....
Ether-extracted cottonseed kernels	.....	.....	.....	.....	25	.....	.....
Cracked corn	65	65	65	65	65	42	40
Wheat bran	10	10	10	10	10	20	20
Milk solids, skim milk, or buttermilk	.....	.....	.....	.....	.....	.....	10
Meat scrap	.....	.....	.....	.....	.....	3	.....
Butter	.....	.....	.....	.....	.....	5	.....
Salt mixture	.....	.....	.....	.....	.....	14	.....
Approximate protein content (N × 6.25)	16.3	16.8	19.5	16.0	19.7	18.7	20.5

(5) The object of feeding cottonseed kernels (diet 5) was for comparison with what might be expected of cottonseed meal in view of its protein and ash content. This diet is practically identical with the diets of lots 1 and 2, except for the toxic factor, which is greatly diminished but probably not entirely removed by extraction with ether.

We may also call attention to the fact that this diet contained, on a basis of nitrogen content, the equivalent of approximately 35 per cent of cottonseed meal.

Seven lots of three pigs each were fed in pens bedded with wood shavings. The pigs were Berkshires about 6 months old.

The composition of the diets is given in Table VIII.

The salt mixture supplied to lot 6 consisted of 350 gm. of calcium lactate (dry basis), 100 gm. of sodium chlorid, and 30 gm. of ferric ammonium citrate to each 100 pounds (45.35 kilos) of feed.

The pigs were fed on diminished rations at first. The amounts fed were gradually increased until the pigs received all they would eat readily twice daily in the form of slop. The animals were supplied with city tap water.

On the sixth day of the experiment it was necessary to cut down the ration of the pigs in lots 1 and 2. And from this time on, these pigs showed increasing dislike for their feed. Frequently at this time pigs in lot 2 (short-cooked meal) were observed by the feeder to vomit after eating. Finally, lots 1 and 2 were consuming only 5 pounds of feed per day as compared with 8 to 10 pounds for the other lots. This behavior was naturally reflected in the weight records. The animals in the other cottonseed-meal lots maintained excellent appetittes and showed good gains for a much longer period.

Table IX gives the data on the feeds consumed by the various lots.

TABLE IX.—*Feed consumption (in pounds) by swine fed cottonseed products*

Period.	Cottonseed meal 1 long-cooked (lot 1).	Cottonseed meal 2 short-cooked (lot 2).	Soybean meal (lot 3).	Peanut meal (lot 4).	Ether-extracted cottonseed kernels (lot 5).	Long-cooked cottonseed meal, supplemented. Lot 6.	Long-cooked cottonseed meal, supplemented. Lot 7.
1–20 days	136.50	135.50	148.00	148.00	148.00	148.00	148.00
21–40 days	149.50	116.50	214.00	212.00	168.50	208.50	205.50
41–56 days	78.00	76.50	157.50	157.50	139.50	148.50	141.00
41–60 days			201.50	201.50	178.50	178.00	167.00
61–83 days			276.00	276.00	228.00	103.90	4.00
Total feed	[a]364.00	[a]328.50	[b]839.50	837.50	723.00	638.90	524.50
Total protein concentrates	91.30	82.10	209.90	209.40	180.80	191.70	178.20
Average per pig	30.30	27.40	69.90	69.80	60.30	63.90	52.50
Average food per day for first 56 days	6.39	5.80	9.31	9.28	8.51	9.37	8.83

[a] Amount offered. Lots 1 and 2 frequently left much of their food.
[b] The smallest pig in the soybean lot was slaughtered on the seventieth day, following an accident by which its backbone was broken at the eleventh dorsal vertebra. For convenience, it is assumed that in lot 3 three pigs were continued at the same rate to the end of experiment.

The pigs fed cottonseed meal received the feed up to the time definite symptoms appeared, and until it was evident that the survivors would not live if continued on the diet. The first death occurred in lot 1 on the forty-seventh day, No. 3, followed shortly by two deaths in lot 2, No. 4, 5 (fig. 4). The survivors in lots 1 and 2 were then eating very poorly and were more or less sick, so that these feeds were terminated on the fifty-sixth day.

The gains made in each lot per pig are given in Table X.

The claim that cottonseed meal "injury," or so-called poisoning, in pigs, is caused by dietary deficiencies or by erroneous practices in feeding seems to be without weight, in view of the comparatively excellent results obtained with pig diets containing ether-extracted cottonseed kernels. Comparing the chemical composition of diets containing corn and cottonseed meal with one containing corn and ether-extracted cottonseed kernels, we see that the protein and mineral factors are kept identical. Possible objection might be raised that the vitamines are either destroyed or pressed out into the oil in the manufacture of the meal. This does not seem to be the case. The temperatures reached by the seed in the manufacture of cottonseed meal are very little if any over 100° C., although the material is in a container whose walls are much hotter than this. This higher temperature of the walls of the cooking drums is maintained by steam under 20 to 50 pounds' pressure. Temperatures taken of material fresh from the drums show apparently a constant limit of 100°, owing to the fact that the kernels are moist, partly from their own or added moisture, which evaporates and thus keeps the temperature from rising.

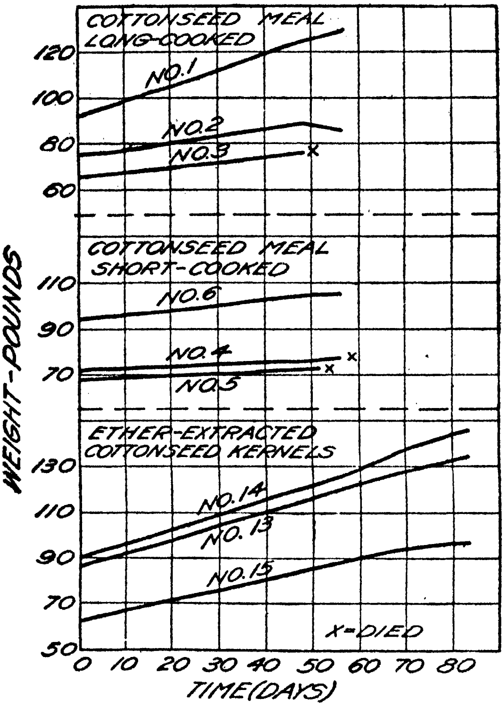

FIG. 4.—Graphs showing the effect of cottonseed products on the growth of pigs. The toxicity of long-and short-cooked meals is quite similar, the pigs showing a gain in weight for a time, but they were either sick or dead in 56 days. The pigs fed on ether-extracted cottonseed kernels continued to thrive throughout the entire period, proving conclusively the removal of the toxic substance by the ether.

TABLE X.—*Effect of various rations on the growth of pigs*

Ration.	Average daily gain per pig. 48 days.	56 days.	70 days.	83 days.	Percentage gain in 56 days is to initial weight.	Total gain in 48 days.	Feed eaten. Total.	Per pound of gain	Number of deaths.	Day on which death occurred.
	Pound.	*Pound.*	*Pound.*	*Pound.*		*Lbs.*	*Lbs.*			
Cottonseed meal 1, long-cooked (lot 1).	0.368	0.335	......	......	21.7	53	327	6.1	1	47
Cottonseed meal 2, short-cooked (lot 2).	.167	.170	......	......	11.9	24	291	12.1	2	52, 56
Soy-bean meal (lot 3).	.646	.678	0.722	0.70	50	93	431	4.6	0	......
Peanut meal (lot 4)..	.555	.588	.633	.655	47	80	431	5.4	0	......
Ether-extracted cottonseed kernels (lot 5)................	.583	.583	.571	.55	41.4	84	380	4.5	0	......
Cottonseed meal 1, butter, salts, meat scrap (lot 6).......	.701	.702	......	......	48.7	101	417	4.1	2	56, 75
Cottonseed meal 1, milk powder (lot 7).	.799	.782	......	......	53.9	115	419	3.6	2	52, 59

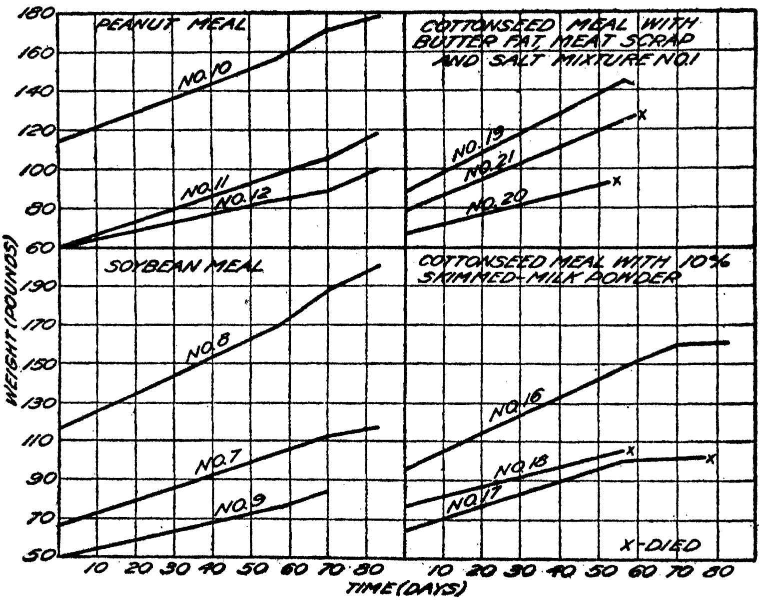

FIG. 5.—Graphs showing the effect of various diets on the growth of pigs. Pigs fed on peanut meal and soybean meal show a decided and regular gain in weight, while those fed with cottonseed meal supplemented by other feed made good gains only during the early part of the feeding period. The toxicity of the meal is shown by the death of four of the pigs and the sickness of the others before the end of the time.

Whether the fat-soluble vitamines pass into the crude oil to any extent is not known. McCollum (*5, 6*) has shown that ether extraction of many seeds does not remove it, possibly as it is a combination not

soluble in ether. Probably the same holds true of oil extraction. McCollum has found that the refined oil lacks the growth-promoting constituent but the refined oil has been treated in ways which might remove this constituent. Richardson and Green (*12*) state that the ether extract of cottonseed flour contains this fat-soluble growth-promoting factor.

TABLE XI.—*Weight record of pigs on various diets*

Lot No.	Feed compared.	Animal No.	Weight. Initial.	Weight. Final.	Weight. Gain.	Gain.	Remarks.
			Lbs.	*Lbs.*	*Lbs.*	*Per ct.*	
1	Cottonseed meal, long-cooked.	1	92. 0	128. 0	36. 0	......	Sick 56th day.
		2	75. 0	86. 5	11. 5	......	Do.
		3	66. 0	76. 0	10. 0	....	Died 47th day.
	Average	....	77. 7	96. 8	19. 2	24. 7	
2	Cottonseed meal, short-cooked.	4	72. 0	76. 5	4. 5	......	Died 56th day.
		5	68. 0	73. 0	5. 0	......	Died 52d day.
		6	93. 0	105. 5	12. 5	......	Sick 56th day.
	Average	....	77. 7	85. 0	7. 3	9. 4	
3	Soybean meal	7	67. 0	118. 0	51. 0	......	
		8	117. 0	200. 5	83. 5	......	
		9	50. 0	83. 5	33. 5	......	Slaughtered.
	Average	....	78. 0	134. 0	56. 0	71. 8	
4	Peanut meal	10	114. 0	179. 0	65. 0	......	
		11	60. 0	118. 0	58. 0	......	
		12	60. 0	100. 0	40. 0	......	
	Average	....	78. 0	132. 3	54. 3	69. 6	
5	Ether-extracted cottonseed kernels.	13	86. 0	133. 0	47. 0	......	
		14	90. 0	146. 0	56. 0	......	
		15	62. 0	96. 0	34. 0	......	
	Average	....	78. 7	125. 0	45. 7	58. 0	
6	Cottonseed meal, long-cooked, butter, meat scrap, and salt mixture.	16	96. 0	161. 0	65. 0	......	Sick 75th day.
		17	65. 0	102. 0	37. 0	......	Died 76th day.
		18	77. 0	106. 0	29. 0	......	Died 56th day.
	Average	....	78. 7	123. 0	43. 7	55. 5	
7	Cottonseed meal, long-cooked, plus 10 per cent milk powder.	19	88. 0	143. 0	55. 0	......	Sick 62d day.
		20	68. 0	93. 0	39. 0	......	Died 52d day.
		21	79. 0	127. 0	48. 0	......	Died 59th day.
	Average	....	78. 3	121. 0	44. 0	56. 2	

The chief chemical difference between cottonseed meal and ether-extracted cottonseed kernels seems to be, then, the presence of gossypol, or the decomposition products of gossypol, to which cottonseed-meal poisoning in swine appears to be due.

It will be noted (fig. 5) that pigs receiving the meal in the supplemented diets ate much more meal and made the best average gains.

With one exception they lived considerably longer than the pigs on unsupplemented diets. None of the pigs which died from the effects of the meal were in poor nutrition, while the pigs of lots 6 and 7, at death, were in normal nutrition. (Table XI.) If we accept the criterion suggested by Wells and Ewing (*14, p. 22*), that—

if well nourished animals die of the injury as is claimed the same cannot be due to a deficient diet and inanition, but must be due to a toxic effect,

then the results of these pig experiments should render nonvalid their conclusion that—

Cottonseed meal injury is due in large part to inadequate diets.

It is doubtful, however, whether this criterion may be generally applicable to deficiency diseases—for example, to beriberi. While the growth curves for small experimental animals, especially fowls and rats, on deficient diets show steep downward slopes to absolute failure, the authors are not aware of cases analogous to those which we report with swine on cottonseed-meal diets (lots 6, 7, Table XI)—namely, the sudden death while gaining rapidly in weight and while in normal control of the limbs. (See also fig. 5, No. 16–21.)

The hypothesis proposed by Rommel and Vedder that cottonseed-meal poisoning in pigs is beriberi, caused by a deficiency in the ration, can not be supported in the terms of our present day ideas of the requirements of an adequate diet. For example, it is evident that our diet 6 is well supplied with the water-soluble and the fat-soluble vitamines, the former having been shown to occur in abundance in cottonseed meal and in corn, while the only moderate deficiency of the fat-soluble accessory in these seeds is overcome by the addition of 5 per cent butter to the diet. The addition of a salt mixture containing calcium, sodium, and chlorin, to supplement these least abundant of the necessary mineral elements in the seeds of plants eliminates the possibility of a deficiency of mineral (matter) as the cause of failure.

As stated before, diets 1 and 2 were discontinued after deaths began to occur in these lots and when the surviving pigs were so noticeably sick that death seemed imminent.

Of the two pigs which survived in lots 6 and 7, one lost in weight and the other made no further gain after discontinuance.

Post-mortem examination of all the pigs which died from cottonseed "injury" showed uniformly the characteristic symptoms associated with the disease—viz, congestion and edema of the lungs, hydrothorax, hydropericardium, etc. The pigs which were slaughtered at the close of the 83 days' experiment were in good condition, and no pathological lesions were evident.

AN EXPERIMENT WITH COMMERCIAL COLD-PRESSED MEAL

Experiments with rats and rabbits revealed the fact that cold-pressed meals were not so markedly toxic as certain hot-pressed meals made from very dry seed and insufficiently cooked. It was thought desirable to ascertain how a cold-pressed meal would affect pigs.

Two pigs in pens were fed for 30 days on a cold-pressed meal and corn meal (1 : 2). The pigs were fed as much as they would readily eat. They consumed relatively much more meal than was eaten during the previous experiments with hot-pressed meal, and maintained good appetites throughout. No safe conclusion may be drawn, but it was evident that these animals were less affected in this short period than the pigs on diets 1 and 2 of the previous experiments in a similar period. The fact that by far the greater part of the gossypol of the raw seed is removed from cold-pressed cottonseed meal and that the remaining small amount may undergo oxidation, etc., while the hot meal is exposed to the air should prove of interest in the practical matter of avoiding cottonseed-meal "injury" of swine. The results of this experiment are given in Table XII.

TABLE XII.—*Effect of commercial cold-pressed cottonseed meal on the growth of pigs*

Pig No.	Weight.			Average quantity of cottonseed meal eaten.	
	Initial.	Final.	Gain.	Total.	Per day.
	Pounds.	*Pounds.*	*Pounds.*	*Pounds.*	*Pounds.*
22	70	85	15	32.1	1.07
23	85	99	14	32.1	1.07

GENERAL SUMMARY AND CONCLUSIONS

Various cottonseed products, including raw cottonseed kernels, ether-extracted kernels, gossypol, and several meals, have been fed to rats, rabbits, poultry, and swine.

Raw cottonseed kernels and the gossypol therefrom have been found highly toxic to all these animals. Cooking the kernels under oil-mill conditions causes a profound reduction in toxicity. This change is so great that the thoroughly cooked products show no pronounced toxic effect on rats and poultry in suitable diets. Thoroughly cooked meals, however, appear to be definitely injurious to rabbits and swine, which are peculiarly susceptible to cottonseed-meal "injury." Rats and fowls are able to withstand much larger relative amounts of cottonseed meal for longer periods. In the "cold-pressing" process of making cottonseed meal the toxic substance passes into the oil to a great extent, thus leaving a meal which may be less harmful than certain hot-pressed meals.

RATS

Cottonseed meal, cottonseed flour, and ether-extracted raw cottonseed kernels have been fed to rats under comparable conditions. Rats fed on extracted kernels have shown superior growth over those on cottonseed meal or cottonseed flour. From this fact it is inferred that even in well-cooked products there remains something slightly deleterious to rats fed on diets containing these as the sole source of vitamines, protein, and minerals. Diets containing well-cooked cottonseed products, with a small amount of milk powder, appear to be as efficient for rats as the control milk diet.

The degree of toxicity of cottonseed meals depends on the thoroughness of cooking in the oil mill. This change appears to be due to oxidation of the gossypol to a substance which we have called "D-gossypol." Some meals may be much more toxic than others, through failure to complete this change. Since evidence shows that the gossypol of the raw seed may be entirely changed to this far less toxic material, it is suggested that the highly toxic effect of the raw cottonseed be described as cottonseed poisoning and that injury due to the meal be described as cottonseed-meal poisoning or injury.

Diets containing cottonseed meal with corn meal, or soybean meal with corn meal, as the sole source of nutriment have led to failure of our rats. The addition of calcium lactate, sodium chlorid, and butter tends to avert this failure.

RABBITS

Rabbits are much more susceptible than rats to cottonseed-meal poisoning. They have been very quickly affected by much smaller relative amounts of the meal in diets which are apparently adequate for these animals.

POULTRY

Aside from an apparently diminished egg production, excessive amounts of cottonseed meal have not appeared to be very injurious to hens. Some evidence is presented to show that the presence of unchanged gossypol in the diet may cause a peculiar discoloration of the egg yolk.

PIGS

Pigs have been fed on diets designed to compare the effect of cottonseed meals with similar protein concentrates, such as peanut meal soybean meal, and ether-extracted cottonseed kernels.

Unsuccessful attempts have been made to avert cottonseed-meal "injury" by supplementing a thoroughly cooked meal with (a) meat scrap, calcium lactate, sodium chlorid, and butter fat, or (b) 10 per cent of skim-milk powder.

Cottonseed meal exerts on pigs a harmful effect, which is not averted by improving the diet with efficient food materials. Such a harmful effect is not produced by similar feedstuffs. Hence, we conclude that the cottonseed-meal "injury" of swine is due, not to deficient diets, but to the presence of a toxic substance. In our opinion this toxic substance in cottonseed meal is the derivative of gossypol which we have called "D-gossypol."

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VARIATIONS IN THE MOISTURE CONTENT OF THE SURFACE FOOT OF A LOESS SOIL AS RELATED TO THE HYGROSCOPIC COEFFICIENT[1]

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INTRODUCTION

In this paper we report a study of the variations in moistness of the various inch sections of the surface foot of soil in some fields near Lincoln, Nebr., during seasons which were exceptionally favorable to the development of both the driest and the moistest conditions ordinarily encountered there.

The moisture content of the soil may be reported as either the total amount present, the free water—the difference between the total water and the hygroscopic coefficient, the growth water—the difference between the total water and the wilting coefficient, the latter value being computed from the moisture equivalent, the hygroscopic coefficient, or some other physical constant (*6, p. 72*)[2]—or, as in the present paper, in a form which makes evident the relative moistness of the soil, as well as all the above-mentioned values, by stating both the hygroscopic coefficient and the relation of the moisture content to this.[3] Thus, the expression 10 × 1.7 would indicate a total moisture content of 17.0 per cent, a wilting coefficient of 15.0,[4] 7.0 per cent of free water, and 2.0 per cent of growth water.

Variations in the moisture content of the surface soil might be expected to increase in importance with increasing humidity of climate, but even in arid regions on lands with the water table far below the surface any important changes in the moistness of the subsoil, other than reductions effected by plant roots or by percolation, appear to be almost entirely dependent upon preceding changes in the moisture content of the surface stratum of soil (*2*), which for the purposes of the present discussion we may consider to extend to a depth of 12 inches.

[1] The work reported in this paper was carried out in 1910 and 1912 at the Nebraska Agricultural Experiment Station, where the authors were, respectively, Chemist and Research Assistant in Chemistry.

[2] References made by number (italic) to "Literature cited," p. 480.

[3] ALWAY, F. J., MCDOLE, G. R., and TRUMBULL, R. S. RELATION OF THE MINIMUM MOISTURE CONTENT OF THE SUBSOIL OF PRAIRIES TO THE HYGROSCOPIC COEFFICIENT. To be published in Botanical Gazette.

[4] 14.7, to be exact.

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A statement of the average moisture content of the surface foot as a whole may be very misleading. Thus, at a time when the average for the whole foot section indicates a fair amount of available moisture, the soil of the first 3 or 4 inches may be too dry to permit germination of seeds, and at another time the optimum condition may be found in the first few inches, while in the lower portion of the section the soil may be too dry to permit the penetration of roots. Therefore detailed moisture studies of the surface soil during periods of exceptionally dry weather are of special interest.

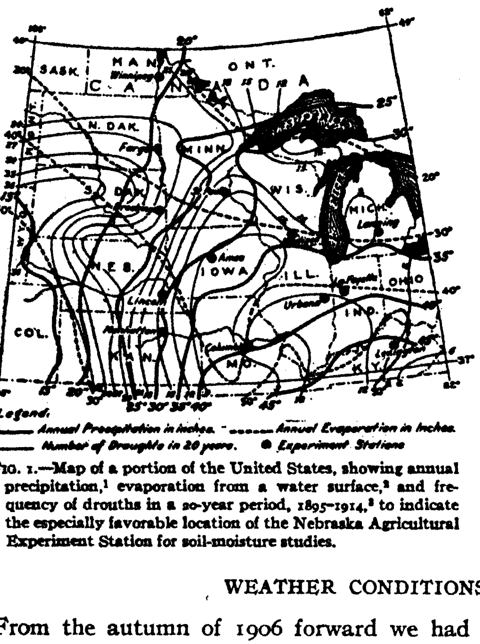

FIG. 1.—Map of a portion of the United States, showing annual precipitation,[1] evaporation from a water surface,[2] and frequency of drouths in a 20-year period, 1895–1914,[3] to indicate the especially favorable location of the Nebraska Agricultural Experiment Station for soil-moisture studies.

For such studies the Nebraska Experiment Station Farm at Lincoln probably provides as good a place as is to be found anywhere in the portion of the United States to be considered as strictly humid, as it lies almost as far to the west as the strictly humid climate extends on the American prairies (fig. 1).

WEATHER CONDITIONS

From the autumn of 1906 forward we had watched at Lincoln for weather conditions that would develop a very dry surface soil, but not until late in the summer of 1909 did these appear, and then at a time when sampling could not be undertaken. They ceased just before meeting the definition of a drouth as used by the United States Weather Bureau: Thirty consecutive days between March 7 and September 30 without a total precipitation of 0.25 inch in 24 hours (7, *chart*). Then ensued a remarkably wet autumn, to be followed in turn by a record-breaking period of dry weather, which was terminated by May rains. We secured some sets of samples during this, but during the dry periods of the following year (1911) we failed to get any. Then we decided upon a detailed study of the moisture variations in the season of 1912, no matter whether it opened wet or dry, beginning the sampling as soon as the frost was out of the ground and taking samples at such times as would

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[2] KIMBALL, H. H. EVAPORATION OBSERVATIONS IN THE UNITED STATES. *In* Mo. Weather Rev., v. 32, no. 12, p. 558. 1904.

[3] NATIONAL WEATHER AND CROP BUL., 1915, NO. 7, 4 p., 4 maps. 1915.

insure our finding the extremes occurring during the season. The maximums could be obtained after heavy rains, but in order to make sure of finding the minimums, it was necessary, whenever the soil began to get dry, to sample at frequent intervals until the next rain restored a moist condition. It happened that the season of 1912 proved almost as satisfactory for such a study as any during the 20-year period beginning with 1895.

TABLE I.—*Nitrogen and organic carbon in different inch sections of the surface foot of the different fields sampled in 1912*

NITROGEN

Depth.	Average of 5 prairie fields.	Grass field. J.	Grass field. M.	Cornfield and fallow F-C.	Exposed subsoil.
Inches.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	0.347	0.403	0.237	0.237	0.047
2	.279	.258	.235	.218	.047
3	.259	.241	.235	.205	.045
4	.245	.236	.233	.200	.044
5	.234	.228	.253	.200	.044
6	.223	.227	.251	.193	.041
7	.210	.212	.239	.182	.040
8	.201	.202	.211	.173	.038
9	.193	.197	.188	.171	.038
10	.181	.190	.171	.164	.038
11	.173	.178	.164	.152	.038
12	.163	.170	.154	.142	.036
Average:					
1-3	.295	.301	.236	.220	.046
4-6	.234	.230	.246	.198	.043
7-9	.201	.204	.213	.175	.039
10-12	.172	.179	.163	.153	.037
1-6	.264	.265	.241	.209	.044
7-12	.186	.191	.188	.164	.038
1-12	.225	.228	.215	.186	.041

CARBON

3	3.31	2.78	2.61	2.18	0.25
8	2.39	2.27	2.27	1.92	.18
12	1.89	1.86	1.52	1.42	.18

CARBON-NITROGEN RATIO

3	12.8	11.6	11.2	10.6	5.6
8	11.9	11.2	10.8	11.1	4.7
12	11.6	10.9	9.7	10.0	5.0

CHARACTER OF SOIL

The soil of all the fields involved in the study is a silt loam of loessial origin, the Marshall silt loam of the Bureau of Soils of the United States Department of Agriculture the properties of which we have discussed[1] in

[1] ALWAY, F. J., McDOLE, G. R., and TRUMBULL, R. S. op. cit.

detail in other articles (*1,4*). In Table I there is reported the nitrogen content of the different inch sections of the fields sampled in 1912, and, for the purposes of comparison, also that of the virgin prairies in the vicinity. The samples used for analysis were composites of equal weights from all the samples on which moisture determinations had been made throughout the season; and thus, for example, from 14 borings in the case of the grass field J and from 36 in the fallow. The data on the prairies represent composites from 250 borings, 50 from each of 5 fields (*1, p. 206*). As the ratio of organic carbon to nitrogen does not change greatly in passing from the first to the twelfth inch in prairies (*1, p. 231*), or even in the ordinary cultivated fields, as may be seen from Table I, the variations in nitrogen may safely be assumed to be accompanied by corresponding variations in the content of organic matter. The low nitrogen content and low carbon nitrogen ratio characteristic of the subsoils is shown in the samples from all levels of the exposed subsoil.

TABLE II.—*Hygroscopic coefficients of the inch sections from the different fields*

Depth.	Grass field J.			Grass field M.			Corn and fallow field.					Exposed subsoil.		
	Set I.	Set II.	Average.	Set I.	Set II.	Average.	Set I.	Set II.	Set III.	Set IV.	Average.	Set I.	Set II.	Average.
Inches.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	10.1	9.5	9.8	8.4	7.4	7.9	8.7	8.5	8.8	7.9	8.5	12.7	12.5	12.6
2	9.0	8.9	9.0	8.0	......	8.0	8.5	8.2	8.7	8.6	8.5	12.7	12.6	12.6
3	9.0	8.6	8.8	8.1	......	8.1	8.5	8.9	9.3	9.0	8.9	12.8	13.0	12.9
4	8.5	8.5	8.5	7.8	7.9	7.9	8.2	8.5	9.2	9.0	8.7	13.2	13.2	13.2
5	8.8	8.4	8.6	8.0	8.5	8.3	8.5	8.7	8.7	9.2	8.8	13.2	12.6	12.9
6	9.0	8.8	8.9	8.1	8.6	8.4	8.7	8.7	9.2	9.5	8.8	13.2	12.5	12.9
7	8.8	9.6	9.2	8.9	9.0	9.0	8.8	9.4	9.3	8.0	8.9	12.8	12.6	12.7
8	8.9	9.5	9.2	9.0	9.6	9.3	8.7	9.9	10.5	8.4	9.4	12.7	12.6	12.7
9	8.5	9.4	9.0	9.1	9.7	9.4	9.3	9.6	10.5	9.2	9.7	12.7	12.7	12.7
10	8.7	10.0	9.4	9.7	10.3	10.0	10.3	10.0	11.2	9.3	10.2	12.3	12.4	12.4
11	9.2	10.7	10.0	10.1	11.1	10.6	11.1	10.5	11.6	9.8	10.8	12.4	12.7	12.6
12	9.1	10.7	9.9	10.0	12.5	11.3	11.2	11.3	12.6	10.5	11.4	12.2	12.9	12.6
Average:														
1 to 6	9.1	8.8	9.0	8.1	......	8.1	8.5	8.6	9.0	8.7	8.7	13.0	12.7	12.9
7 to 12	8.9	10.0	9.4	9.5	10.3	9.9	9.9	10.1	11.0	9.2	10.1	12.5	12.6	12.6
1 to 12	9.0	9.4	9.2	8.8	......	9.0	9.2	9.4	10.0	9.0	9.4	12.7	12.7	12.7

No analyses were made of the samples taken in 1910, but these were from fields very similar to the grass field and fallow mentioned in Table I.

In the case of 1910 samples the hygroscopic coefficient was determined for each sample in which the moisture content was obtained, but in 1912 we confined this determination to four sets from one field and two from each of the others, each sample being a composite of two borings (Table II). While in the case of the portion of the foot section below the reach of the plow it would have been far better to have had a determination of the hygroscopic coefficient of each sample, the amount of labor involved made this prohibitive. The results from the duplicate or quadruplicate sets are sufficiently concordant to make it appear probable that in using the averages of these we introduce no serious errors. In the case of fields M and F–C, in which the surface soil through cultivation had been

kept well mixed, we might use even a single value for the first 6 sections, as we do in Table V. Even in the old grass field J and in the exposed subsoil the hygroscopicity does not vary widely from inch to inch of the lower half of the foot section.

The soils involved in this study are well represented by the Lincoln surface soil D and subsoil A, which we have used in various laboratory studies involving the movement of water (*2, p. 32; 3, p. 399*), the former being taken from various parts of the 10-acre field F-C and the latter from an adjacent excavation.

The variation in density from field to field is, as we have previously pointed out (*1, p. 224*), so great as to make data on the apparent specific gravity desirable. In June, 1912, we took from each of the fields two sets of composite samples, each from five borings, using the 4-inch plate auger mentioned below. Considerable variations in density (Table III) are shown, but scarcely sufficient to necessitate the use of different values in computing from the moisture percentages the equivalent in inches of rain. While the exposed subsoil was the most dense of all, the especially high values found for the surface inch of this are to be attributed to the beating effect of the rains. In the bluegrass pasture there was no distinct variation from level to level, except that the first inch was much the least dense. The surface layer of 6 or 7 inches in the cornfield was distinctly lighter than the lower portion of the foot section.

TABLE III.—*Apparent specific gravity of the soil at the different levels*

Depth.	Bluegrass pasture.			Cornfield.			Exposed subsoil.		
	Set I.	Set II.	Average.	Set I.	Set II.	Average.	Set I.	Set II.	Average.
Inches.									
1	0.87	0.81	0.84	1.35	1.20	1.28	1.63	1.94	1.81
2	1.17	1.19	1.18	1.09	1.07	1.08	1.42	1.38	1.40
3	1.23	1.29	1.26	1.13	1.16	1.15	1.35	1.38	1.37
4	1.22	1.28	1.25	1.18	1.02	1.10	1.33	1.44	1.41
5	1.16	1.25	1.21	1.15	1.03	1.10	1.49	1.56	1.52
6	1.16	1.21	1.19	1.09	1.11	1.10	1.32	1.51	1.42
7	1.10	1.20	1.16	1.11	1.10	1.11	1.42	1.48	1.45
8	1.16	1.24	1.20	1.14	1.29	1.22	1.40	1.52	1.45
9	1.19	1.21	1.20	1.27	1.32	1.29	1.37	1.37	1.37
10	1.21	1.22	1.22	1.32	1.35	1.33	1.32	1.39	1.36
11	1.24	1.25	1.24	1.33	1.34	1.34	1.37	1.35	1.46
12	1.24	1.25	1.24	1.36	1.34	1.35	1.25	1.42	1.34
Average	1.16	1.20	1.18	1.20	1.20	1.20	1.40	1.48	1.44

CONDITIONS DURING AN EXTREME SPRING DROUTH

WEATHER OF 1910

A striking feature of the weather of 1910 at Lincoln was the record-breaking drouth of the spring. The autumn of 1909 had been unusually wet (Table IV) and favorable for the moistening of the soil, while in

December and January the precipitation was somewhat above normal, and the ground remained frozen from December 4 until early in February, except for an occasional thaw of the surface inch or two. These conditions favored the retention of the maximum amount of moisture which the soil could hold against gravity. Then followed 94 days, January 29 to May 2, in which the total precipitation amounted to only 0.30 inch at Lincoln, and to 0.22 at the Nebraska Experiment Station, this being the driest 3-month period shown by the meteorological record for Lincoln, beginning in 1881. February was normally cold, but at the end of the month the temperature rose rapidly and during March continued high, the mean for the latter month being 16 degrees above the normal. April also was warm, with a mean of 4 degrees above the normal. The wind movement throughout this dry period was not unusually great, but during March and April the proportion of sunshine was much higher and the relative humidity of the atmosphere much lower than normal.

TABLE IV.—*Daily precipitation at the Nebraska Experiment Station from November 1, 1909, to May 31, 1910*

Day.	November.	December.	January.	February.	March.	April.	May.
1	1.25	0.75					
2		.52					0.40
3		.24	0.14				
4		.07					
5							.87
6		.12					.52
7							.21
8							
9					0.08		
10							
11	.14						
12			.52				
13	2.93	.04					
14	1.11		.02			0.01	
15							.10
16							.33
17						.04	
18							
19							
20				0.09			
21	.25						.24
22							
23							
24							
25							
26							
27							.14
28	1.57	.28					.34
29			.20				
30	.75						.15
31							
Total	8.00	2.02	.88	.09	.08	.05	3.30

The absence of precipitation after January, together with the abnormally high temperatures of March, caused an unusually early drying of the surface soil. At the end of the first week in April evidences of drouth had become very marked, and on the 9th we sampled three fields to a

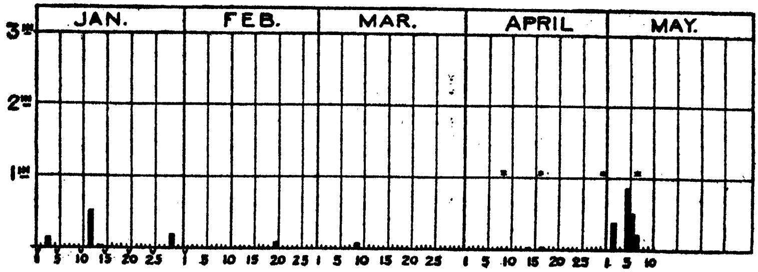

FIG. 2.—Diagram showing daily precipitation at Lincoln, Nebr., during the latter part of the record-breaking drouth of the spring of 1910. The dates of sampling are indicated by asterisks.

depth of 6 inches: a fall-plowed cornfield, a bluegrass pasture, and an alfalfa field that had been seeded in 1907. These fields were sampled weekly until the drouth was ended in May by 2 inches of rain, and once immediately after this fall, as shown in figure 2.

TABLE V.—*Moisture conditions in the surface 6 inches of soil during the spring of 1910*

MOISTURE CONTENT

Depth.	Bluegrass pasture.				Alfalfa field.				Fall-plowed field.			
	Apr. 9.	Apr. 16.	Apr. 30.	May 7.	Apr. 9.	Apr. 16.	Apr. 30.	May 7.	Apr. 9.	Apr. 16.	Apr. 28	May 7.
Inches.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	9.9	7.3	8.0	38.1	9.4	9.0	4.7	35.8	4.2	6.9	5.8	39.6
2	14.4	10.3	9.8	30.8	16.3	12.9	9.0	31.7	20.0	25.4	21.4	33.1
3	12.5	11.5	10.8	31.3	16.6	12.1	10.2	32.5	26.6	26.2	21.6	37.5
4	12.7	11.8	10.9	30.9	16.9	15.5	14.5	31.3	25.8	29.0	23.1	34.7
5	13.0	12.8	10.9	31.0	17.3	16.1	14.6	30.2	31.6	31.3	24.5	34.5
6	13.5	13.4	11.0	30.5	17.5	16.3	20.7	30.7	31.5	30.3	26.0	34.4
HYGROSCOPIC COEFFICIENT												
1–6	9.5	9.5	9.5	9.5	9.2	9.2	9.2	9.2	10.9	10.9	10.9	10.9
RATIO OF MOISTURE CONTENT TO HYGROSCOPIC COEFFICIENT												
1	1.0	0.8	0.8	4.0	1.0	1.0	0.5	3.9	0.4	0.6	0.5	3.6
2	1.5	1.1	1.0	3.2	1.8	1.4	1.0	3.4	1.8	2.3	2.0	3.0
3	1.3	1.2	1.1	3.3	1.8	1.3	1.1	3.5	2.4	2.4	2.0	3.4
4	1.3	1.2	1.1	3.3	1.8	1.7	1.6	3.4	2.4	2.7	2.1	3.2
5	1.4	1.3	1.1	3.3	1.9	1.8	1.6	3.3	2.9	2.9	2.2	3.2
6	1.4	1.4	1.2	3.2	1.9	1.8	2.3	3.3	2.9	2.8	2.4	3.2

MOISTURE CONDITIONS

The moisture conditions in the three fields are reported in Table V. The hygroscopic coefficients of the various inch sections of the different sets were determined, but in calculating the ratios, etc., we have used a

single value for each field—the average of all the determinations on the samples from that field—as in each field the differencess between the various samples were less than the experimental error. The samples were composites of sections from 12 cores taken 5 to 10 feet apart with a 1.5-inch soil tube.

At the time of the first sampling, the soil of the bluegrass field was already dry, showing a ratio of only 1.3 to 1.5 During the following three weeks it became steadily drier until, on April 30, the ratio had fallen to 1.1. The rains following this raised it to an average of 3.4.

Throughout the dry period the soil in the alfalfa field was distinctly moister than that in the grass field, but here also it became steadily drier until the May rains raised the ratio to an average of 3.5.

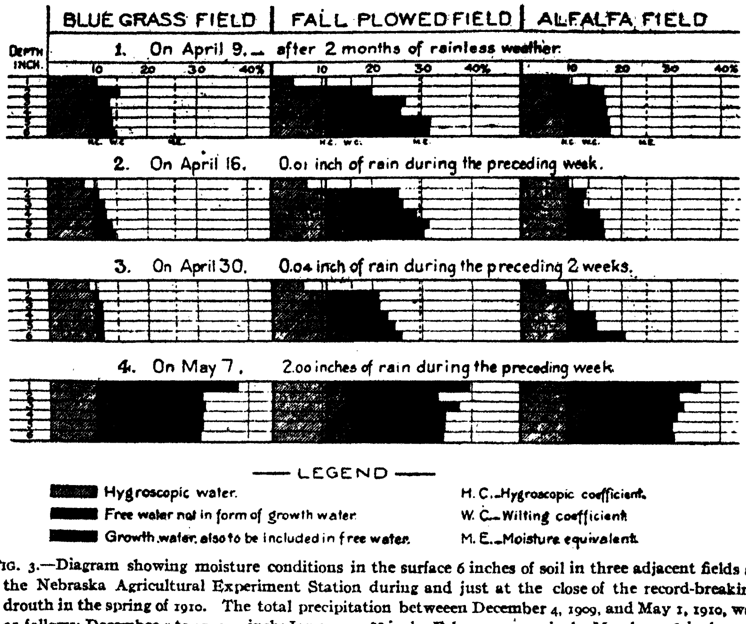

FIG. 3.—Diagram showing moisture conditions in the surface 6 inches of soil in three adjacent fields at the Nebraska Agricultural Experiment Station during and just at the close of the record-breaking drouth in the spring of 1910. The total precipitation betweeen December 4, 1909, and May 1, 1910, was as follows; December 5 to 31, 0.44 inch; January, 0.88 inch; February, 0.09 inch; March, 0.08 inch; and April, 0.05 inch.

In the fall-plowed field the moisture in the surface inch was already at the minimum on the occasion of the first sampling, the ratio being only 0.4. During the following week of dry weather it did not change appreciably, but during the next 12 days the average ratio in the 3-to-6-inch section fell to 2.2. Even then the moisture conditions below the first inch were favorable for plant growth. The May rains raised the ratio to an average of 3.2.

While the loss of moisture in the fall-plowed field was due entirely to evaporation, the uniform loss in moisture in the sections below the

second inch in the grass field indicates that the loss in it was due to transpiration, except for the slight amount lost from the first 2 inches. In the alfalfa field, with its more open stand of plants, the losses due to evaporation appear to have been greater and to have affected the soil to a greater depth.

Only in the surface inch did we find a ratio lower than 1.0. In the fall-plowed field, which had been disked in March, the surface 2 inches of soil were drier on April 9 than 19 days later.

The moisture relations are shown graphically in figure 3. The values for the wilting coefficients and moisture equivalents used in this have been computed from the hygroscopic coefficients (6, *p. 72*).

TABLE VI.—*Weather conditions at Lincoln, Nebr., in the season of 1912 compared with the normal*

	March.	April.	May.	June.	July.	August.
PRECIPITATION (INCHES)						
In 1912 [a]	2.06	2.23	0.69	4.03	2.68	4.15
Normal	1.23	2.77	4.25	4.32	3.83	3.71
Departure	.83	−.54	−3.56	−.29	−1.15	.44
MEAN TEMPERATURE (°F.)						
In 1912	26	53	66	68	79	75
Normal	36	51	63	72	76	74
Departure	−10	2	3	−4	3	1
SUNSHINE (PERCENTAGE OF POSSIBLE)						
In 1912	56	62	83	68	79	74
Normal	68	66	66	73	76	74
Departure	−12	−4	17	−5	3	0
WIND VELOCITY (MILES PER HOUR)						
In 1912	10	14	13	9	11	9
Normal	13	14	12	10	9	9
Departure	−3	0	1	−1	2	0
RELATIVE HUMIDITY (PER CENT)						
In 1912	80	64	58	63	61	67
Normal	70	63	68	69	67	71
Departure	10	1	−10	−6	−6	−4

[a] At University Farm.

CONDITIONS THROUGHOUT AN UNFAVORABLE SEASON

WEATHER OF SEASON OF 1912

The weather of the crop season of 1912 as a whole, as may be seen from Table VI, did not depart widely from the normal, but the practically rainless month of May with a hot wind near its close was very unfavorable for winter wheat, meadows and pastures. This dry period was, in so far as the winter wheat crop was concerned, the most severe we had an opportunity to observe during our seven years' connection with the Nebraska Experiment Station. The 30-day period, April 22 to May 31, with a total precipitation of 1.07 inches would have fallen within the definition of drouth mentioned above, except for the rain of 0.32 inch on May 4.

TABLE VII.—*Daily precipitation at the Nebraska Experiment Station from March 1 to August 31, 1912*

Day.	March.	April.	May.	June.	July.	August.
1	Trace.	0.01	0.10	0.50		
2	0.50				0.16	0.04
3	Trace.					
4			.32	.10		
5	Trace.					1.30
6		.08		Trace.		
7	.01					.04
8	.03			.30		.07
9				.21	.24	
10	.08		.15			
11	.33	.05		.06	.81	
12				.36	.05	
13	.26			2.35		.06
14	.43			.10		
15					.04	.26
16				.02		1.85
17						
18					.38	
19	Trace.					.21
20	.42	.95	.02		.25	
21		.76				
22						
23	Trace.					
24						
25		.07				
26			.10		.60	
27					.15	
28		.19				
29		.12		.03		
30						
31	Trace.					.32
Total	2.06	2.23	.69	4.03	2.68	4.15

The precipitation and temperatures of the last four months of 1911 had been more favorable than normal to the accumulation of moisture

in the soil. In the months of January and February together the precipitation amounted to 1.38 inches, compared with a mean of 1.32 inches, while the first three weeks of March were both wetter and colder than normal. The last of March was warm and dry, and by April 4 the frost was out of the ground in most fields, a little being found only in grass fields. None was met with in any of the later samplings. April did not depart much from the normal in temperature, rainfall, or wind movement.

May was very unfavorable for crops, the rainfall of 0.69 inch, occurring in five light showers (Table VII), being only one-sixth of the normal, while the latter half of the month was marked by very high temperatures, which on some days were accompanied by high winds. The most unfavorable day was the 26th, when the temperature rose to 98° F.; from

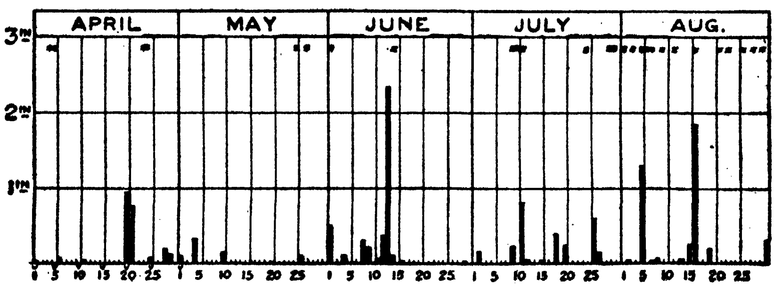

FIG. 4.—Diagram showing daily precipitation at Lincoln during the season of 1912. The dates of sampling are indicated by asterisks.

11 a. m. to 9 p. m. the wind velocity averaged 31 miles per hour, and the temperature 94°, a phenomenon locally described as a "hot wind."

The early part of June was dry but cool and cloudy. A heavy rain (2.71 inches) on the 12th and 13th of the month was followed by three weeks of practically rainless weather. This period of low atmospheric humidity, almost cloudless skies, and normal temperatures approached a drouth. July and August did not depart widely from the normal, but dry periods appeared in both months.

For the purposes of a soil-moisture study the season was exceptionally favored by the two dry periods, April 30 to May 30 and June 14 to July 11, and the two heavy rains of June 13 and August 15 and 16. The weather conditions preceding the various samplings are summarized in Table VIII. (See also fig. 4.)

FIELDS SAMPLED

All the fields sampled in this season were close together and also to the Experiment Station buildings, the most distant, M, being less than a quarter of a mile from the University Farm rain gage.

TABLE VIII.—*Weather conditions in 1912 at the Nebraska Experiment Station preceding the various samplings*

Date of sampling.	Fields sampled.[a]	Weather conditions of interval since previous sampling.
Apr. 4	J	The preceding three months had been colder than normal, with a normal amount of precipitation. Frost had disappeared, except a little within the surface, 6 inches of J. All the precipitation of March (2.06 inches) had been in the form of snow.
5	F–C	
23	J	Normal weather; 1.84 inches of rain since Apr. 5, 1.71 inches of this being on the 20th–21st instant.
24	F–C	
May 25	J and F–C	Very dry month, with only 0.97 inch in seven light showers. Average temperature and wind velocity not far from normal, but with an excess of sunshine.
27	S	0.10 inch rain on 26th.
June 1	J and F–C	A dry, hot, windy week, with a hot wind on the 26th. A rain of 0.50 inch had ended a few hours before the samples were taken.
14	J, F, and S	A dry, cool, cloudy fortnight, ending in a rain of 2.71 inches on June 12–13.
July 9	S	An almost cloudless, rainless three-week period, with very dry atmosphere but normal temperatures.
10	F	A shower of 0.24 inch late in the day before.
11	F and S	In forenoon 0.81 inch of precipitation, much of it in the form of hail. Samples taken in afternoon.
24	J	Temperatures, wind, and sunshine only slightly above normal, but rainfall only 0.72 inch, with the last (0.25 inch) on the 20th.
29	J	0.75 inch of rain on July 26–27. Other factors normal.
30	F and S	
Aug. 1	C and S	Normal weather. No rain.
3	C, F, M, and S	Cool, calm, cloudy; 0.04 inch of rain on Aug. 2.
5	M and S	Cool weather; 1.30 inch of rain earlier in the day.
6	C, F, and M	Normal weather. No rain.
7	C and F	Normal weather; 0.04 inch of rain.
9	C, F, M, and S	Cool and cloudy, with 0.07 inch of rain on the 8th.
12	C, F, and M	Normal weather. No rain.
16	C, F, and M	Cool and cloudy, with 2.11 inches of rain during the night preceding.
21	C and M	Weather almost normal, with 0.21 inch of rain on the 19th.
23	C, F, and M	Temperature and wind normal, but no rain and skies almost cloudless.
26	C, F, M, and S	A continuation of the preceding, except somewhat warmer.
28	C, F, and M	A continuation of the preceding.
30	F and M	Do.

[a] F=fallow field; C=cornfield; F–C=cornfield before corn plants were large enough to have an appreciable effect upon soil moisture; J and M=grass fields; S=exposed subsoil.

Field J had been in bluegrass and used as a pasture for many years. The amount and distribution of nitrogen in the first foot of this (Table I) were very similar to that in virgin prairies near by; the chief difference lay in the first inch, in which the bluegrass field showed the higher values. As this field was not under the control of the Experiment Station, and the frequent samplings were interfering with its use, it was found necessary at the end of July to abandon it. For the continuation of the study

use was made of a small part of field M, in which bluegrass had almost entirely crowded out the alfalfa sown in 1907. Previous to that year the field for almost 40 years had been in annual crops. However, the moisture data indicate that the change caused no serious break in continuity, as on August 3 the moisture conditions in field M were very similar to those in J on July 29, except for the losses from the surface few inches during the interval.

As a representative area of both fallow and corn land we employed a field lying between fields J and M and referred to below as C–F. After a long period in bluegrass it had been plowed a few years before. In 1911 it was in corn, and after the removal of the crop had been fall-plowed. In the spring of 1912 the whole of it had been planted to corn, but on June 14, in order to provide a fallow area for the study, we hoed out all the plants on a tract 6 rods square, and after this had the plot cultivated along with the rest of the field. It was kept entirely free of weeds, thus serving as a summer fallow, while the rest of the field was typical of corn land. In the latter the samples taken up to that time between the corn rows may be regarded as quite representative of fallow as well as of corn land.

The fourth area, a tract of exposed subsoil, S, lay beside a new interurban line, in preparing the grade for which the soil had been removed to a depth of 3 or 4 feet. Throughout the crop season we kept this exposed subsoil free of weeds by hoeing, but gave it no other cultivation. With each heavy shower there was a great loss from run-off, as the surface was hard and had a very gentle slope, while on the other tracts, which were almost level, there was practically none.

All the places in fields J, C–F, and S where samples were taken were sufficiently far from trees and alfalfa plants to avoid any draft upon the soil moisture by roots, but this was not the case in field M which was surrounded by alfalfa, and itself carried a few alfalfa plants.

The moisture conditions within the surface foot are shown in Tables IX to XII, while Table XIII indicates the general conditions in the underlying subsoil.

TABLE IX.—*Ratio of moisture content to hygroscopic coefficient in the surface foot of grass fields in 1912*

Depth.	April.		May.	June.		July.		August.											Extremes.	
	4	23	25	1	14	24	29	3	5	6	9	12	16	21	23	26	28	30	Maximum.	Minimum.
Inches.																				
1	4.0	4.0	0.7	3.6	4.7	1.1	2.0	1.3	4.1	3.4	2.7	1.4	4.3	3.7	2.6	1.4	1.4	1.1	[a]4.7	0.7
2	3.3	3.3	1.3	2.5	3.4	1.3	2.1	1.4	3.8	3.4	3.0	2.0	4.0	3.5	3.3	1.8	1.8	1.5	4.0	1.3
3	3.4	3.4	1.2	1.9	3.5	1.4	1.8	1.6	3.4	3.1	2.8	2.1	3.8	3.2	2.7	2.1	1.7	1.6	3.8	1.2
4	3.5	3.5	1.4	1.4	3.4	1.3	1.4	1.5	3.0	2.8	2.7	2.0	3.7	3.1	2.8	1.9	1.7	1.5	3.7	1.3
5	3.4	3.5	1.4	1.4	3.6	1.4	1.3	1.3	2.1	1.6	2.3	1.7	3.5	2.8	2.6	1.9	1.7	1.5	3.6	1.3
6	3.3	3.4	1.4	1.3	3.4	1.3	1.2	1.3	1.6	1.3	2.2	1.8	3.5	2.9	2.6	1.9	1.7	1.7	3.5	1.2
7	3.3	3.2	1.3	1.3	3.3	1.4	1.2	1.3	1.4	1.3	2.1	1.8	3.1	2.6	2.5	1.9	1.7	1.6	3.3	1.2
8	3.2	3.2	1.3	1.3	3.5	1.4	1.2	1.3	1.3	1.3	1.8	1.8	2.8	2.5	2.5	1.8	1.8	1.7	3.5	1.2
9	3.2	3.2	1.4	1.4	3.4	1.4	1.2	1.3	1.4	1.3	1.5	1.8	2.7	2.4	2.5	1.8	1.8	1.6	3.4	1.2
10	3.0	3.0	1.6	1.3	3.4	1.4	1.2	1.3	1.3	1.2	1.4	1.8	2.3	2.1	2.1	1.6	1.8	1.6	3.4	1.2
11	2.8	2.8	1.5	1.3	3.1	1.3	1.2	1.3	1.2	1.2	1.4	1.7	1.8	2.0	1.8	1.5	1.7	1.5	3.1	1.2
12	2.8	2.8	1.6	1.4	3.0	1.4	1.3	1.2	1.1	1.1	1.4	1.6	1.4	1.5	1.5	1.6	1.6	1.4	3.0	1.1
Average:																				
1–3	3.6	3.6	1.1	2.7	3.9	1.3	3.0	1.4	3.8	3.3	2.8	1.8	4.0	3.5	2.9	1.8	1.6	1.4	4.0	1.1
4–6	3.4	3.5	1.4	1.4	3.5	1.3	1.3	1.4	2.2	1.9	2.4	1.8	3.6	2.9	2.7	1.9	1.7	1.6	3.6	1.3
7–9	3.2	3.2	1.3	1.3	3.4	1.4	1.2	1.3	1.4	1.3	1.8	1.8	2.9	2.5	2.5	1.8	1.8	1.6	3.4	1.2
10–12	2.9	2.9	1.6	1.3	3.2	1.4	1.2	1.3	1.2	1.2	1.4	1.7	1.8	1.9	1.8	1.6	1.7	1.5	3.2	1.2
1–6	3.5	3.5	1.2	2.0	3.7	1.3	2.1	1.4	3.0	2.6	2.6	1.8	3.8	3.2	2.8	1.9	1.7	1.5	3.8	1.2
7–12	3.0	3.1	1.4	1.3	3.3	1.4	1.2	1.3	1.3	1.2	1.6	1.8	2.4	2.2	2.1	1.7	1.7	1.5	3.3	1.2
1–12	3.2	3.3	1.3	1.7	3.5	1.3	1.4	1.3	2.1	1.9	2.1	1.8	3.1	2.7	2.4	1.8	1.7	1.5	3.3	1.3

[a] The exceptional value of 4.7 was found on June 14 just after a very heavy rain.

TABLE X.—*Ratio of moisture content to hygroscopic coefficient in the surface foot of fallow field in 1912*

Depth.	April.		May.	June.		July.			August.										Extremes.	
	5	24	25	1	14	10	11	30	3	6	7	9	12	16	23	26	28	30	Maximum.	Minimum.
Inches.																				
1	2.7	3.6	0.5	2.6	3.9	1.8	3.4	2.1	2.7	3.6	3.5	2.2	2.0	4.0	2.4	1.7	1.9	1.3	4.0	0.5
2	3.2	3.5	1.1	2.0	3.8	1.1	3.2	2.7	2.4	3.5	3.4	2.7	2.7	3.9	2.8	2.4	2.5	2.2	3.9	1.1
3	3.2	3.4	1.8	1.3	4.0	1.6	2.9	2.8	2.4	3.4	3.4	2.9	3.0	3.9	3.0	2.7	2.6	2.6	4.0	1.3
4	3.4	3.7	2.6	1.8	4.1	2.3	3.0	3.0	2.9	3.4	3.4	3.1	3.2	3.9	3.2	3.0	2.8	2.8	4.1	1.8
5	3.5	3.6	2.4	2.7	3.7	3.0	3.2	3.1	3.0	3.5	3.4	3.1	3.1	3.9	3.5	3.2	2.9	2.8	3.9	2.4
6	3.5	3.7	2.9	3.0	3.7	3.1	3.3	3.3	3.3	3.4	3.5	3.1	3.1	3.9	3.4	3.1	3.1	3.0	3.9	2.4
7	3.5	3.5	3.0	3.0	3.8	3.2	3.3	3.2	3.3	3.2	3.3	3.2	3.2	3.7	3.3	3.1	3.0	3.0	3.8	3.0
8	3.3	3.6	2.9	3.1	3.5	3.3	3.1	2.8	3.1	2.9	3.1	2.9	2.9	3.3	3.0	2.8	2.8	2.8	3.6	2.8
9	3.1	3.2	2.8	2.9	3.4	2.9	2.8	2.7	2.8	2.9	2.9	2.8	2.8	3.1	2.9	2.7	2.7	2.6	3.4	2.6
10	2.8	2.9	2.5	2.6	3.3	2.6	2.7	2.6	2.6	2.6	2.7	2.6	2.6	2.9	2.7	2.6	2.6	2.6	3.3	2.5
11	2.6	2.7	2.4	2.5	2.9	2.5	2.5	2.5	2.6	2.4	2.5	2.5	2.5	2.7	2.6	2.5	2.5	2.5	2.9	2.4
12	2.5	2.5	2.3	2.3	2.7	2.4	2.4	2.3	2.4	2.3	2.4	.....	2.4	2.6	2.4	2.4	2.4	2.4	2.7	2.3
Average:																				
1–3	3.0	3.5	1.2	1.7	3.9	1.5	3.2	2.5	2.5	3.5	3.4	2.6	2.6	3.9	2.4	2.3	2.3	2.0	3.9	1.2
4–6	3.5	3.7	2.6	2.5	3.8	2.6	3.2	3.1	3.1	3.4	3.4	3.1	3.1	3.9	3.4	3.1	2.9	2.9	3.9	2.5
7–9	3.3	3.5	2.9	3.0	3.6	3.1	3.1	2.9	3.1	3.0	3.1	3.0	3.0	3.4	3.1	2.9	2.8	2.8	3.6	2.8
10–12	2.6	2.7	2.4	2.5	3.0	2.5	2.5	2.5	2.5	2.4	2.5	2.5	2.5	2.4	2.6	2.5	2.5	2.5	3.0	2.4
1–6	3.2	3.6	1.8	2.1	3.8	2.1	3.2	2.8	2.8	3.0	3.4	2.8	2.8	3.9	2.9	2.7	2.6	2.4	3.9	1.8
7–12	2.9	3.1	2.6	2.8	3.3	2.8	2.8	2.7	2.8	2.7	2.8	2.7	2.8	2.9	2.8	2.7	2.6	2.6	3.3	2.6
1–12	3.1	3.3	2.3	2.5	3.5	2.5	3.0	2.7	2.8	3.1	3.1	2.8	2.8	3.4	2.9	2.7	2.6	2.5	3.5	2.3

TABLE XI.—*Ratio of moisture content to hygroscopic coefficient in surface foot of cornfield during August, 1912*

Depth.	August—											Extremes.	
	1	3	6	7	9	12	16	21	23	26	28	Maximum.	Minimum.
Inches.													
1	2.0	1.3	3.9	3.3	2.4	1.8	4.1	3.3	2.5	1.6	1.2	4.1	1.2
2	2.3	1.6	3.6	3.1	2.7	2.4	4.0	3.2	2.6	2.1	1.6	4.0	1.6
3	2.3	1.7	3.2	2.9	2.5	2.3	3.7	2.9	2.5	2.1	1.8	3.7	1.7
4	2.4	1.7	3.0	2.7	2.4	2.4	3.7	3.1	2.6	2.3	2.0	3.7	1.7
5	2.2	1.7	2.6	2.3	2.1	2.2	3.6	3.0	2.6	2.2	2.1	3.6	1.7
6	2.2	1.7	2.1	2.2	1.9	2.1	3.0	3.0	2.6	2.4	2.0	3.0	1.7
7	2.3	1.8	2.0	2.0	1.9	2.0	2.7	2.7	2.5	2.4	2.0	2.7	1.8
8	2.3	1.8	1.8	1.9	1.9	1.9	2.5	2.4	2.4	2.2	1.9	2.5	1.8
9	2.4	1.8	1.8	1.9	1.8	1.9	2.2	2.2	2.1	2.1	1.9	2.4	1.8
10	2.3	1.8	1.8	1.9	1.8	1.9	1.9	2.1	2.0	1.9	1.8	2.3	1.8
11	2.2	1.8	1.8	1.9	1.8	1.8	1.8	2.0	1.9	1.7	1.7	2.2	1.7
12	2.1	1.7	1.7	1.8	1.8	1.8	1.7	1.8	1.8	1.6	1.6	2.1	1.6
Average:													
1–3	2.2	1.5	3.5	3.1	2.5	2.2	3.9	3.1	2.5	1.9	1.5	3.9	1.5
4–6	2.3	1.7	2.6	2.4	2.3	2.2	3.4	3.0	2.6	2.3	2.0	3.0	1.7
7–9	2.3	1.8	1.9	1.9	1.9	1.9	2.5	2.4	2.3	2.2	1.9	2.5	1.9
10–12	2.2	1.8	1.8	1.9	1.8	1.8	1.8	2.0	1.9	1.7	1.7	2.2	1.7
1–6	2.2	1.6	3.0	2.7	2.4	2.2	3.6	3.0	2.5	2.1	1.8	3.0	1.6
7–12	2.2	1.8	1.8	1.9	1.8	1.8	2.2	2.2	2.1	1.9	1.8	2.2	1.8
1–12	2.2	1.7	2.4	2.3	2.1	2.0	2.9	2.6	2.3	2.0	1.8	2.9	1.7

TABLE XII.—*Ratio of moisture content to hygroscopic coefficient in surface foot of bare exposed subsoil in 1912*

Depth.	May.	June.	July.			August.						Extremes.	
	27	14	9	11	30	1	3	5	9	16	26	Maximum.	Minimum.
Inches.													
1	0.7	2.3	0.4	1.9	1.0	0.7	0.9	1.8	1.6	2.1	1.2	2.4	0.4
2	1.2	2.4	.6	1.6	1.6	1.4	1.2	1.9	1.8	2.2	1.8	2.4	.6
3	1.7	2.4	.9	1.4	1.6	1.7	1.6	1.7	1.8	2.0	1.9	2.4	.9
4	1.8	2.3	1.4	1.7	1.9	1.8	1.9	1.8	1.9	2.0	2.0	2.3	1.4
5	2.1	2.3	1.8	1.9	2.0	2.0	2.0	1.9	2.0	2.1	2.0	2.3	1.8
6	2.1	2.3	1.8	2.1	2.0	2.2	2.0	2.0	2.0	2.2	2.0	2.3	1.8
7	2.1	2.3	2.0	2.2	2.0	2.2	2.1	2.0	2.1	2.2	2.0	2.3	2.0
8	2.1	2.3	2.1	2.1	2.0	2.2	2.1	2.1	2.1	2.2	2.0	2.3	2.0
9	2.1	2.4	2.2	2.2	2.1	2.2	2.1	2.2	2.1	2.2	2.0	2.4	2.0
10	2.2	2.5	2.2	2.3	2.2	2.3	2.2	2.1	2.2	2.1	2.1	2.5	2.1
11	2.0	2.4	2.2	2.2	2.1	2.2	2.2	2.1	2.2	2.1	2.1	2.4	2.1
12	1.9	2.4	2.2	2.3	2.2	2.3	2.2	2.2	2.2	2.1	2.1	2.4	2.1
Average:													
1–3	1.2	2.4	.6	1.6	1.4	1.3	1.2	1.8	1.7	2.1	1.6	2.4	.6
4–6	2.0	2.3	1.7	1.9	2.0	2.0	2.0	1.9	2.0	2.1	2.0	2.3	1.7
7–9	2.1	2.3	2.1	2.2	2.0	2.2	2.1	2.1	2.1	2.2	2.0	2.3	2.0
10–12	2.0	2.4	2.2	2.3	2.2	2.3	2.2	2.1	2.2	2.1	2.1	2.4	2.0
1–6	1.6	2.3	1.1	1.7	1.7	1.6	1.6	1.8	1.9	2.1	1.8	2.3	1.1
7–12	2.0	2.3	2.1	2.2	2.1	2.2	2.2	2.1	2.1	2.1	2.0	2.3	2.1
1–12	1.8	2.3	1.6	1.9	1.9	1.9	1.9	2.0	2.0	2.1	1.9	2.3	1.8

TABLE XIII.—*Moisture conditions in subsoil of the various fields in 1912*

Depth.	Fallow.			Grass field.				Cornfield.			Exposed subsoil.			
		Ratio.		J.		M.			Ratio.			Ratio.		
	Hygroscopic coefficient.	June 19.	Aug. 29.	Hygroscopic coefficient.	Ratio June 19.	Hygroscopic coefficient.	Ratio Aug. 29.	Hygroscopic coefficient.	July 9.	Aug. 29.	Hygroscopic coefficient.	June 19.	July 9.	Aug. 29.
Feet.														
2	14.7	1.2	1.8	14.3	1.5	14.3	1.2	13.6	1.6	1.4	12.2	2.4	2.2	2.2
3	13.5	1.6	1.8	13.6	1.5	13.6	1.2	13.7	1.7	1.4	12.2	2.4	2.2	2.2
4	13.0	1.9	1.7	13.3	1.6	13.3	1.2	13.6	1.2	1.5	11.8	2.5	2.3	2.4
5	13.0	1.9	1.8	13.3	1.9	13.3	1.2	13.5	1.7	1.9	12.0	2.6	2.3	2.4
6	[a] 13.0	2.1	2.0	13.2	2.1	13.2	1.2	13.3	1.8	2.0	[a] 12.0	2.7	......	2.4

[a] Datum missing. Value assumed.

The fallow, grass field J, and exposed subsoil were sampled to a depth of 6 feet on June 19, using composites of three cores taken 10 to 15 feet apart with a soil tube. On July 9 the cornfield and exposed subsoil were sampled, and on August 29 the fallow, grassfield M, cornfield, and exposed subsoil. The data on the hygroscopic coefficients of only the samples taken on July 9 and those from the fallow on August 29 are available, the other samples through an oversight being thrown out before the determinations could be made. So, in order to compute the ratios, we have in the case of three fields, F, C, and S, used the single set of coefficients on each, and in that of field M the average of those for F and C.

METHOD OF SAMPLING

The samples were obtained by means of a 4-inch plate auger with a shield, made especially for the purpose. An iron tube of 4 inches inside diameter was driven 6 inches into the ground and the auger worked inside this. It carried an adjustable 6-inch shield which could be raised 1 inch at a time and fastened by a setpin, thus automatically guarding against more than 1 inch of soil being removed without resetting the pin. The fields were sampled at two places, 10 to 20 feet apart, and the samples from the duplicate sets combined. The soil from each of the twelve 1-inch sections was placed in a covered can as soon as removed from the ground, thus preventing loss by evaporation between the time it was removed from the ground and its weighing.

EXTREMES IN MOISTNESS

The extremes in moistness were shown by the fallow and the grassfields, the cornfield occupying an intermediate position, while the exposed subsoil with its lower water-retaining capacity in comparison with

its hygroscopic coefficient, its finer texture (Table II), and its almost negligible content of organic matter (Table I), behaved quite differently from the three others.

UPPER LIMIT

At the time of the first sampling the frost was out of the plowed land and almost gone from the grass fields. Not more than a trace of rain had fallen within a 24-hour period during the 16 days preceding this, but still the ratios in both the grass field and the fallow were found almost as high as at any time later in the season, except immediately after very heavy rains, as on June 14 and August 16. In the two fields mentioned the ratios were similar, except for the drier condition of the surface inch in the fallow, and averaged alike, 3.1, for the 11-inch section (2 to 12 inches), and almost alike, 3.2 and 3.1, respectively, for the whole 12-inch section. At the second sampling, 19 days later, the ratios were almost the same, with an average of 3.3 in both fields. A rain of 1.71 inches had fallen three days before.

The heaviest rain of the season, 2.81 inches in 48 hours, fell on June 12 and 13. Samples were taken from all three fields within less than 12 hours after the cessation of this, and the highest ratios of the season found, averaging 3.7 and 3.9 for the surface 6 inches and 3.5 for the surface 12 inches of the grass field and fallow. The surface inch in the former showed the exceptionally high value of 4.7. In laboratory experiments such high ratios have been found to be common where the downward movement of the water is delayed (*2, p. 40*).

The second heaviest rain, 2.11 inches, fell during the night of August 15–16. Early in the following forenoon the uppermost 9 inches in the fallow was found as moist as on June 14, and that in the grass field almost as moist, but the lower part of the foot was distinctly drier.

In the cornfield ratios practically as high as those in the fallow were found near the surface when the sampling followed soon after a rain, as on August 5, 7, and 16, but the high ratios did not extend so far from the surface, and this for the simple reason that the corn kept withdrawing water from all levels, while in the fallow the loss was confined to evaporation through the surface, and during the month of August the rains were not sufficient to restore the moisture content of the lower sections in the cornfield to their water-retaining capacity.

On the exposed subsoil the maximum ratios were found on June 14; but even then, when the samples had been taken only a few hours after the cessation of a heavy rain, the highest ratio was 2.4 or 2.5, and the average for the 12 inches 2.3, compared with 3.5 in both fallow and grass field. While the compact, smooth, weedless surface with a gentle slope increased the run-off and prevented the ready penetration of water, this relatively low maximum, found also in laboratory experiments with similar soil (*soil A, 3, p. 402*), must be attributed to the character of the soil itself rather than to the surface conditions.

Thus the highest ratios found in the fallow and grass fields soon after heavy rains were 3.6 to 4.0 in the uppermost 6 inches and 2.9 to 3.3 in the second 6 inches, and under the influence of percolation alone these fell gradually to 2.5 to 3.0 in the lower half-foot and to a slightly higher minimum in the upper 6-inch section. In the exposed subsoil there was no difference to be observed between the upper and lower halves of the foot section, both showing a ratio of 2.3 to 2.5 soon after heavy rains, and this gradually falling to 2.1 or 2.2.

LOWER LIMIT

Marked differences, owing to the withdrawal of water through the roots in the grass and corn fields, developed during periods of dry weather, and continued even after moderate rains.

In the fallow loss of moisture could occur only through evaporation from the surface or percolation into the subsoil, with the result that there was a much greater loss from the levels near the surface. The extreme condition was shown on May 25, when the ratio in the surface inch fell to 0.5 and in the second inch to 1.1, while in the fourth it was still 2.6. The same limited depth of drying was shown during the dry periods preceding the samplings of July 10 and August 30. The persistent moistness throughout the greater portion of the surface foot in the fallow may be well brought out by considering the second 6-inch section as a whole. The ratio in this did not fall below 2.6 at any time during the five months included in the study, and on only three occasions, May 25, August 28 and 30, was it found this low. The ratio for this 6-inch section varied only between the limits of 2.6 and 3.1, except immediately after the very heavy rain of June 12–13. Even in the surface layers the ratio was in general well above that corresponding to the wilting coefficient (1.5), in the fourth inch on no occasion falling below 1.8, and in the third inch being found below 1.6 only once, on June 1. Even in the second inch it was below 2.0 for only very short periods.

There appears no reason to suspect that during the part of the year not included in the study we could have found drier soil in any of the levels than we encountered in the course of the work, which embraced the greater part of the growing season. From this it is evident that at Lincoln in fields kept in clean cultivation only rarely does the second inch of soil become too dry to permit the germination of seeds and the satisfactory growth of roots, while the fourth inch appears to remain sufficiently moist for these purposes throughout even exceptionally severe drouths.

The conditions in the cornfield were found intermediate between those in the fallow and those in the grass fields. Between June 14 and August 1 no samplings were made among the corn plants. After the plants had become large enough to make an appreciable draft upon the soil moisture,

the loss from below the first few inches was, like that in the grass field, quite uniform, as may be seen from the data for August 3 and 28; but the ratios did not fall as low, 1.7 being the minimum. Whether a severe drouth near the end of the growing season would have induced in the cornfield ratios as low as those found in the grass field earlier in the season can not be decided from the data, but it should be pointed out that in the latter part of August, when the corn was making the heaviest demands upon the soil moisture, the ratio in the lower half of the surface foot of even the grass field did not fall below 1.5, while on August 28 it was 1.8 in the cornfield, compared with 1.7 in the grass field.

On the exposed subsoil, with a smooth compact surface without a soil mulch, such as existed in the fallow, the effect of evaporation extended a little deeper, a ratio as low as 1.4 being found in the fourth inch on July 9, and ratios below 1.0 in the first three 1-inch sections. Below the fourth inch the ratios were quite uniform throughout the season from level to level, the average for the second 6-inch section varying only between 2 and 2.2, except after the heavy June rain, when it rose to 2.3. In general the ratio was 0.6 or 0.7 lower than that for the corresponding section in the fallow.

DEPTH OF PENETRATION OF RAINS

The depth of penetration of the water from different rains (Table XIV) is indicated by the distance from the surface to which the ratios were increased. In the fallow this was only roughly proportional to the amount of rain, but here the moistness of the soil below the uppermost 3-inch section was comparatively constant. Only two rains were sufficiently heavy, those of June 12–13 and August 16, to affect the twelfth inch and only the first of these two gave any evidence of having caused an addition of moisture to the second foot of soil. The low ratios found on August 29 in the levels below the first foot (Table XIII) support this conclusion. The June rain caused the passage of water into the second foot in the grass field also, but that of August 16 affected the moistness to only 10 inches. The much lesser penetration of the various rains in the exposed subsoil is to be attributed to the run-off from the smooth, hard, gently sloping surface before much of the water had time to enter the soil.

The actual addition of water to the soil of the first foot, as computed from the increase in moisture content and the relative density of the soil (Table III), amounted to nearly 90 per cent of the rainfall in the case of the grass fields, but to only about half as much in the fallow. Most of this difference must be regarded as an actual loss through run-off, as in the case of only one rain, that on June 12–13, can any part of it be attributed to a portion of the water having passed through the first into the second foot.

TABLE XIV.—*Depth of penetration and addition of moisture to the surface foot of soil*

Date of sampling.	Rainfall.	Interval between cessation of rain and sampling.	Maximum penetration.			Water added to surface foot by the rain.		Approximate ratio in fallow before rain.	
			Fallow.	Grass field.	Exposed subsoil.	Fallow.	Grass field.	1 to 3 inches.	4 to 6 inches.
	Inches.	*Hours.*	*Inches.*	*Inches.*	*Inches.*	*Inches.*	*Inches.*		
July 10	0.24	24	1	......	......	......	......	1.1	2.6
June 1	.50	10	2	2 or 3	2	0.3	0.5	1.2	2.6
July 11	.81	4	6 or 7	......	2	.7	......	1.5	2.6
Aug. 5	1.30	6	6	7	3	.4	1.1	2.5	3.1
Aug. 16	2.11	6	11 or 12	10	3	.9	1.8	2.6	3.1
June 14	2.71	24	[a]12	[a]12	[a]12	1.4	2.5	1.5	2.4

[a] Rain on this occasion penetrated beyond the maximum depth of sampling: 12 inches.

The effect of a rain in increasing the moisture content of a fallowed field depends not only upon the amount of rain and the rapidity of its fall but also upon the moistness of the surface soil. Thus, an inch of rain that has fallen just slowly enough to avoid all run-off may have penetrated during the first 24 hours only 4 or 5 inches into a silt-loam soil when the initial ratio was only 0.5, whereas it would have penetrated twice as far had the initial ratio been 1.5 (*3, p. 402–403*). The half-inch rain of June 1 was held within the surface 2 inches, a comparatively dry layer in the third inch separating this upper partly moistened section from the already moist soil below (fig. 5).

LOSSES THROUGH EVAPORATION

It is evident that in the grass fields, and during August in the cornfield, the losses of water from the levels below the surface 3-inch section took place almost entirely through transpiration. In the fallow, when the ratios were as high as 2.8 to 3.3, there would be a slow downward movement of moisture into the subsoil if this were less moist until the ratio had fallen to some point between 1.9 and 2.4 (*2, p. 50*), while at the same time there would be a slow upward movement until the ratios in the sections at some little distance from the surface had fallen to about the same point. In cylinder experiments with surface soil from the fallow field it was found with two sets of 3-foot cylinders which had been filled with soil having initial ratios of 2.4 and 3.0, respectively, that at the end of 78 days the lower half of the surface foot showed ratios of 1.9 and 2.1, respectively. From other laboratory experiments it is evident that losses through evaporation from the portions of the soil 6 inches or more below the surface take place very slowly after the ratio has once been reduced to 2.0. From both laboratory experiments (*2, p. 50*) and field observation (*2, p. 63*), it is evident that the downward movement of

moisture becomes practically negligible when the ratio has been reduced to 2.0.

In the case of the fields involved in the present study, the effect of evaporation in reducing the ration below 2.0 appears to have been confined to the surface 3 inches in the fallow and exposed subsoil and to a shallower layer in the grass fields, and, during August, in the cornfield.

RELATION OF MINIMUM MOISTURE CONTENT TO WILTING COEFFICIENT AND HYGROSCOPIC COEFFICIENT

The wilting coefficient as defined by Briggs and Shantz corresponds to the ratio 1.47 (6, *p. 65*). In the cornfield during the month of August ratios lower than this were not found, although throughout the season as a whole the moisture supply of soil and subsoil together had been abnormally low, as evidenced by the yield of only 6 tons per acre of silage, and by the data in Table XII. From this it would appear that in the surface foot of cornfields at Lincoln we would rarely find growth water absent, even in seasons of drouth, and that in the case of the corn crop a statement of the amount of growth water would have more significance than that of free water. In the grass fields, on the other hand, while the free water at no time fell below about 2 per cent, the growth water was distinctly below zero on various occasions, and toward the end of July this dry condition persisted in the lower 6-inch section for at least a fortnight. The method of expressing the moisture conditions used above appears to us to have all the advantages of both of these as well as some possessed by neither.

RELATION OF MOISTURE RETENTIVENESS TO CONTENT OF ORGANIC MATTER

In both fallow and grass fields the highest ratios were observed in the surface 7 inches where the proportion of organic matter was highest, and in both these soon after heavy rains. In the eleventh- and twelfth-inch sections the maximums were below 3.0, thus approaching those in the exposed subsoil. The minimums were to be expected near the surface, but a ratio lower than 1.0 was observed only in the first inch, and this only on May 25. In the fallow the minimum ratio below the fourth inch varied between only 2.3 and 3.0, while for the different levels below the first inch in the grass fields it varied between only 1.1 and 1.3, the lower 3-inch sections showing the lowest values.

In the exposed subsoil, almost entirely lacking organic matter, the maximums were much lower. A ratio of 2.0 to 2.2 seems the highest to be expected in this after it has had a few days in which to lose by seepage the excess of water added by rain. Accordingly, ratios of 0.5 to 0.7 and 1.0 to 1.2 would indicate the proportion of growth water and free water, respectively, retained in the exposed subsoil as contrasted with 0.9 to 1.5 and 1.4 to 2.0 in the fields with an ordinary surface soil, rich in organic matter. The same results have been obtained in labora-

tory experiments already reported (No. 2, 3), but which were carried out after this field study had been concluded. The difference is shown in Table XV, compiled from the data in Tables X and XII.

TABLE XV.—*Difference in ratios shown by uncropped surface soil and exposed subsoil, from data for the 7–12-inch section*

Date.	Ratio.			Date.	Ratio.		
	Surface soil.	Subsoil.	Difference.		Surface soil.	Subsoil.	Difference.
May 25, 27	2.6	2.0	0.6	August 3	2.8	2.2	0.6
July 11	2.8	2.2	.6	9	2.7	2.1	.6
30	2.7	2.1	.6	16	2.9	2.1	.8
				26	2.7	2.0	.7

PROPORTION OF RAINFALL ACCUMULATED IN THE FALLOW

With the fallow field it is of interest to know how large a proportion of the total precipitation, 13.78 inches, which fell between the first and last samplings was accumulated in the surface soil and subsoil together. From the data in Table IX it is evident that after only three rains, those of April 20–21 (1.71 inches), June 12–14 (2.81 inches), and August 15–16 (2.11 inches), could any appreciable amount of water have penetrated beyond the twelfth inch. In the case of the last two rains the minimum amounts of water required to raise the ratios from those existing before the rain to those found immediately after must have been approximately 1.4 and 0.8 inch, respectively, thus leaving a maximum of only 4.4 inches which could have passed into the second foot. Moreover, this amount would be possible only on the assumption that there was no run-off, while the data in Table XIV indicate that with the heavy rains the loss by run-off amounted to nearly half of what fell. On all three occasions at the time of the sampling there doubtless was some water in the surface foot which would later have passed into the second foot if evaporation had been prevented, but under the conditions which prevailed it is probable that only a negligible quantity actually did so. On August 30 the first foot of soil contained the equivalent of about 0.8 inch of rain less than it did on April 5. Thus, it appears probable that out of the 13.78 inches of rain which fell during the five months less than 2 inches were accumulated in the fallow.

More frequent cultivation of the surface, with the object of maintaining a dry mulch, would probably have only reduced the amount of water accumulated, as, on one hand, moist soil would have been brought to the surface to lose its water by evaporation, and, on the other, very dry soil, with a ratio below 1.0, would have been brought next the moist layer in the fourth or fifth inch to absorb part of the free water contained in this and retain it as hygroscopic water.

DISCUSSION OF RESULTS

DISTRIBUTION OF AVAILABLE MOISTURE IN SURFACE LAYERS

After prolonged dry weather following good rains the soil without plant cover through the first few inches shows a rapid rise in moistness from the surface downward, while where there is a full stand of plants, as in the grassfields, the rise is slight and a low ratio extends beyond the twelfth inch. Where a moderate rain has fallen after the latter condition has once been established, there will be a high and comparatively uniform degree of moistness through several inches and then a sharp fall, but where very heavy rains have fallen there will be almost uniformly high ratios. These conditions are illustrated by figure 5, showing graphically part of the data from Tables IX, X, and XII.

RELATIVE IMPORTANCE OF SUCCESSIVE SOIL LEVELS AS SOURCES OF MINERAL NUTRIENTS

It is customary in making chemical studies of the soil to distinguish sharply between the 6-to-9-inch portion reached by tillage implements, called the "soil" or "surface soil," and that below, referred to as the "subsurface," or sometimes as the "subsoil." As it has been assumed that nutrients are secured through the roots mainly from the former, this has received the chief attention. As a source of nitrogen the surface layer will be much the more important because of the generally much higher content of nitrogen and the more aerobic conditions found in this.

On the soils of at least the Missouri River territory, such as represented in the present study, it is evident that a similar assumption for the mineral nutrients is not justifiable. The decline in moistness within the surface foot of the grassfields is quite uniform (Table IX), the withdrawal of water appearing as rapid in the lowest 3-inch section as in the second; and there is nothing in the data to suggest that this uniformity does not extend to a considerable distance below the twelfth inch. Almost the same remarks apply to the cornfields after the plants have made their main growth of stalk.

However, the similar readiness with which water is given up to the roots from the two levels specified does not necessarily indicate that they are equally important as sources of mineral nutrients to the crops. Aside from the fact that with annuals these are largely absorbed during the early stages of growth there is the important consideration that on nonirrigated lands the uppermost of the two sections will be in a moist condition a much greater proportion of the time, owing to many of the summer showers not being sufficiently heavy to cause any increase in moistness beyond the first few inches, as is well illustrated in the above tables. Hence, as a source of mineral nutrients the first 6 inches will be

more important than the second, although the depth of the plowline has little to do with the matter.

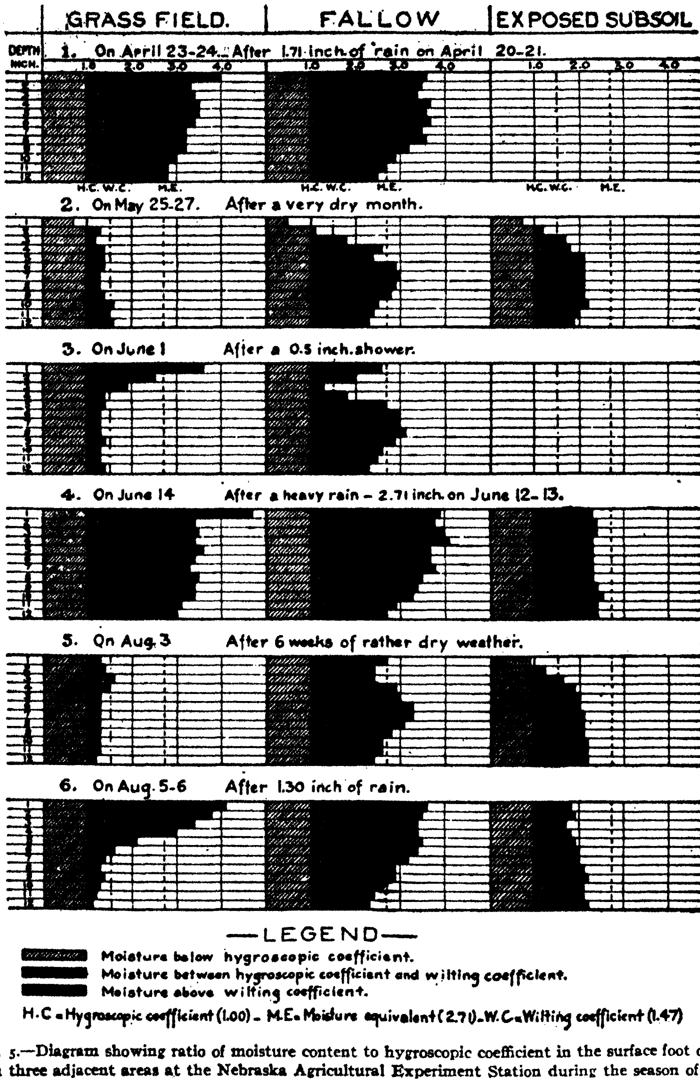

FIG. 5.—Diagram showing ratio of moisture content to hygroscopic coefficient in the surface foot of soil on three adjacent areas at the Nebraska Agricultural Experiment Station during the season of 1912. The data illustrate also the relation of the distribution of moisture to both the plant cover and the preceding weather.

Where the availability of the phosphoric acid and potash in the lower levels is distinctly less than in the plowed layer, the above remarks regarding the mineral nutrients would not apply, but with the loess soils here discussed no such difference in availability has been found (*5, p. 21*).

FREQUENCY OF DROUTHS AS AN INDEX OF THE SEVERITY OF SEASONS

The frequency of drouths as defined above is not an index of the severity of different seasons. In the 20-year period, 1895–1914, fifteen occurred at Lincoln (Table XVI), seven of these during the period of our connection with the Nebraska Agricultural Experiment Station. From the table it will be seen that the drouths so defined are especially likely to begin with the first day of March, or at least to include the greater part of that month. Of the fifteen, seven began on March 1 and two others covered a large part of that month. April is the month in which drouths are next in prominence, while May, June, July, and August have each played an important part in only a single drouth, and September in none. In so far as most crops are concerned the months of real drouth, those showing a marked deficiency in soil moisture as contrasted with a deficiency in precipitation, are June, July, and August. The dry period in May, 1912, which did not meet the above definition of drouth was far more severe on vegetation than any of the seven drouths we had an opportunity to observe. This emphasizes the failure of any single weather factor to indicate satisfactorily a deficiency of soil moisture, or to indicate a drouth in so far as crop growth is concerned.

TABLE XVI.—*Drouths[a] during the crop season, at Lincoln, Nebr., in the 20-year period, 1895–1914*

Series No.	Year.	Period.	Duration.	Chief month of drouth.
			Days.	
1	1895	Mar. 1 to Apr. 28	59	March and April.
2	1895	Apr. 30 to May 30	31	May.
3	1895	June 29 to July 28	30	July.
4	1899	Mar. 12 to Apr. 25	45	March and April.
5	1900	Mar. 1 to Apr. 14	45	March.
6	1902	Mar. 1 to Apr. 24	55	March and April.
7	1903	Mar. 1 to Apr. 9	40	March.
8	1906	Aug. 8 to Sept. 13	37	August.
9	1908	Mar. 6 to Apr. 16	41	March.
10	1909	Mar. 1 to Apr. 5	35	Do.
11	1909	Apr. 7 to May 11	34	April.
12	1910	Mar. 1 to May 1	62	March and April.
13	1911	Mar. 1 to Apr. 25	56	Do.
14	1911	June 8 to July 8	30	June.
15	1912	Mar. 21 to Apr. 19	30	April.

[a] Defined as a period of 30 consecutive days between Mar. 1 and Sept. 30 without a total precipitation of 0.25 inch in 24 hours (7, *chart*).

SUMMARY

The paper reports a study of the variations in moistness of the different inch sections of the surface foot of soil in some fields near the Nebraska Agricultural Experiment Station, which is near, but still within, the western limit of the strictly humid portion of the American prairies. The work was carried out during seasons which were exceptionally favorable

to the development of both the driest and the moistest conditions ordinarily encountered there, and the degree of moistness is expressed as the ratio of the water content of the soil to the hygroscopic coefficient. The soils were loessial silt loams in adjacent fields, including a clean summer fallow, a cornfield, grass fields, and an area of subsoil exposed by grading operations and kept free of vegetation.

The extremes were shown by the fallow and the grass fields. As the frost disappeared from the ground at the close of a fortnight of rainless weather the ratios in both were found as high as at any time later in the season, except immediately after very heavy rains, being alike in the two fields and averaging 3.1 to 3.2. A few hours after the cessation of a rain of 2.8 inches, ratios of 3.7 to 3.9 were found in the surface 6-inch section in both fields, but of only 2.9 to 3.3 in the second 6-inch section.

The lowest ratios in the portions of the foot section below the immediate surface were found in the grass fields, where, during dry periods, they fell as low as 1.2, the twelfth inch becoming as dry, and this as quickly, as the overlying levels, evidence that the chief loss of water was through transpiration. In the cornfield as the plants approached maturity the moisture was withdrawn uniformly from the different levels but the ratios did not fall below 1.5.

In the fallow the soil at only a few inches from the surface remained moist throughout the driest periods. In the second 6-inch section the ratio did not fall below 2.6, varying during the season only between the limits 2.6 and 3.1. Even in the fourth inch the ratio did not fall below 1.8, while in the second it was below 2.0 for only very short periods.

In the grass fields after the ratios had been reduced to a low point only rains amounting to 2.0 inches or more were able to penetrate to a depth of 12 inches, the water from lighter rains being held within the surface foot until it was transpired by the plants or evaporated from the surface. In the fallow with its moist soil the penetration was not much greater, but this is to be attributed to the rain falling more rapidly than it could be absorbed or retained on the plant free surface.

In the grass fields, and in the cornfield after the plants were well grown, the moisture was lost chiefly through transpiration, while on the fallow and exposed subsoil it was lost through evaporation and run-off, but little of the rainfall of the five months covered by the study reaching the second foot. In the grass field doubtless practically all the 13.8 inches that fell were so returned to the atmosphere, while in the fallow probably less than the equivalent of 2.0 inches of rain was accumulated in surface soil and subsoil together.

The exposed subsoil kept free of vegetation differed markedly from the fallow with ordinary surface soil, the maximum ratio being only 2.4 to 2.5 and in the second 6-inch section the ratio during the season varied only between 2.0 and 2.3. The differences in the maximum ratio between the first and the second 6-inch sections in the fields with ordinary surface

soil and the much greater difference between the ratios in the surface layers of these and those in the exposed subsoil appear at least partly due to the differences in the proportion of organic matter.

Under the climatic conditions of the locality more significance is to be attached to a statement of the amount of growth water than that of the free water in the case of corn, while the reverse holds true for grass fields, but neither of these is as satisfactory as a statement of the hygroscopic coefficient, together with the ratio of the moisture content to this.

The distribution of free water in the surface foot assumes characteristic forms, dependent upon the preceding weather conditions and the presence of a plant cover.

The moisture relations indicate that as a source of mineral nutrients the upper half of the surface foot may be more important than the lower, but this is due to the depth of penetration of rains and not to the depth of the plowline, nor to the distribution of the roots.

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SUBSOILING, DEEP TILLING, AND SOIL DYNAMITING IN THE GREAT PLAINS[1]

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GENERAL DISCUSSION OF SUBSOILING, DEEP TILLING, AND SOIL DYNAMITING

There is perhaps no agricultural operation that is so often and enthusiastically advocated and at the same time so little practiced as that of loosening or tilling the soil below the depth reached by the ordinary plow.

The supposed necessity or desirability of such an operation appears to be based on a widespread belief that only that part of the soil loosened and moved by man with his implements of tillage is utilized by nature in the production of crops; that this part of the soil is the only part that participates in the storage of water to be recovered by the crop; that the development and growth of the roots of crop plants is limited to this portion of the soil, and that this is the only portion of the soil from which plant food may be obtained by the crop.

A less extreme belief recognizes that these things are not entirely limited to the portion of the soil that man loosens, stirs, pulverizes, or inverts, but holds that the soil so treated provides a more effective medium for their action than does the undisturbed soil.

Such beliefs apparently either overlook the luxuriant vegetation produced on land that has never known the tillage implements of man or assume that the roots of crop plants are essentially different in their relation to the soil than those of other plants or of the same plants growing wild.

Studies of the root systems of agricultural crops have shown that in the deep soils and subsoils of the prairies and plains the roots of annual crops are well distributed through the soil to a depth of 3 feet or more.

[1] The experimental work of this investigation was carried out at 12 field stations of the Office of Dry-Land Agriculture Investigations. The following members of the scientific staff of the office assisted in the experiments at their stations: J. M. Stephens, superintendent, Judith Basin Substation, Moccasin, Mont., in charge of northern district; O. J. Grace, superintendent, Akron (Colo.) Field Station, in charge of central district; E. F. Chilcott, superintendent, Woodward (Okla.) Field Station, in charge of southern district; W. P. Baird, assistant, Judith Basin Substation; A. E. Seamans, assistant, Huntley (Mont.) Field Station; W. A. Peterson, superintendent, F. E. Cobb and N. O. Henchel, assistant arboriculturists, Max Pfaender, assistant in horticulture, J. T. Sarvis, physiologist, and R. S. Towle, assistant, Mandan (N. Dak.) Field Station; O. R. Mathews, assistant, Bellefourche (S. Dak.) Field Station; F. L. Kelso, superintendent, Ardmore (S. Dak.) Field Station; Albert Osenbrug, assistant, Scottsbluff (Nebr.) Field Station; W. E. Lyness, assistant, Archer (Wyo.) Field Station; J. F. Brandon, assistant, Akron (Colo.) Field Station; A. L. Hallsted, assistant, Hays (Kans.) Substation; C. B. Brown, assistant, Garden City (Kans.) Substation; L. N. Jensen, assistant, Amarillo (Tex.) Field Station; H. G. Smith, superintendent, Tucumcari (N. Mex.) Field Station.

If the water stored within the zone of normal root development is not sufficient to meet the needs of the crop, the roots will continue during the life of the plants to penetrate deeper, provided the soil below is wet. Under such conditions the roots may successively occupy the fourth, fifth, and sixth foot. The roots of winter wheat have been traced to a depth of 8 feet. In this connection it should be noted that fertility of the subsoil is a general characteristic of the soils of semiarid regions. Roots do not penetrate dry soil, even though there may be wet soil beneath it. Where shallowness of soil restricts root development to a depth less than normal, the plants may attain complete development, provided the water content of the zone occupied by the roots is maintained above the limit of availability. The shallower the soil or the smaller the quantity of available water it can retain the more dependent is the crop on rains that fall while it is growing.

All field studies of root systems have been made on land given ordinary plowing, generally to a depth of about 6 inches. No comparative studies of root systems as developed in deep and in shallow plowing have been made. But studies that have been made on the quantity of water stored in the soil, the depth to which it is stored, the depth from which it is used and the degree to which it is exhausted, and the behavior and yield of the crop on land tilled to different depths all afford an abundance of indirect evidence that the form and extent of root systems are not primarily affected by the depth of tillage.

Extensive soil-moisture studies that have been made in connection with the investigations reported in this paper indicate that the ability of the soil to take in or to retain water, or to give up water to the crop, is not determined by the depth of tillage. Sands and light sandy soils offer little resistance to the entry and downward passage of water. They are little changed and certainly not improved in this respect by cultivation. With the heavier clay soils in which penetration is slower and more difficult it would seem that there was more opportunity for improvement by a mechanical loosening. The result is not, however, what it might at first thought appear to be. The mechanical loosening that may be affected when such soils are dry enough to be loosened by tilling is of no consequence so long as the soil remains dry. When rains come and water enters the soil, it carries soil material with it in its downward passage through the loosened soil. The clay expands on becoming wet and the loosened and wetted area becomes an amorphous mass. On drying, the soil contracts. A part of the shrinkage is downward, and a part of it is lateral. The lateral shrinkage results in cracks that may open the soil as effectively as any tillage operation. Mathews (*3*)[1] has shown that when allowed opportunity for free expansion a soil when wet may occupy 2½ times the volume it did when dry.

[1] Reference is made by number (italic) to "Literature cited," p. 521.

One cycle of wetting and drying overcomes the effect of cultivation. As Mosier and Gustafson (4) say—

> The subsoil ran together as soon as it was wet and became approximately as it was before.

It is frequently stated that deep tillage prevents run-off by facilitating penetration. Soil-moisture studies show that in the Great Plains penetration and run-off are determined more by the condition of the immediate surface than by the depth to which the soil has been cultivated. Run-off in the Great Plains other than that from the rapid melting of snow on frozen ground is from torrential rains in which the precipitation of a few minutes or hours is measured in inches. Beating rains of this character smooth and compact the surface so that the run-off is heavy. As the great volume of water that constitutes the run-off does not get beneath the surface, the condition of the subsoil is of no importance in determining its amount. The finer and smoother the surface has been made by cultivation the more easily and quickly is it reduced to a condition that resists penetration and facilitates run-off. The Akron, Colorado, soil, on which the results of subsoiling have been especially unfavorable to the practice, is a good example of a soil from which there often may be heavy run-off from a smooth and compacted surface overlying a very loose and open subsoil.

Many cases have been noted in the course of these experiments where the amount of water that entered the soil from a heavy rain was greater on a dry, cracked stubble than on a plowed field.

Under the semiarid conditions of the Great Plains, where production is determined by the quantity of water available to the crop, the amount of water that enters and is retained by the soil is not determined by the depth of cultivation, and consequently is not increased by an increase in such depth. Under more humid conditions, where rainfall is sufficient to fill the soil regardless of the amount that may be lost by run-off, the depth of cultivation can not add to the amount retained and so can not be a determining factor, in so far as this item is concerned.

It must be recognized, however, that the possible combinations of conditions of looseness, fineness, and water content of subsoil and surface and the character and amount of rainfall are so many that they are seldom exactly duplicated, particularly in semiarid regions. Different combinations of these factors may give rise to different results, as is clearly shown both in soil-moisture studies and in the crop yields reported in this paper. The study of root systems and of soil moisture indicates that the effect or lack of effect of differences in the depth of tillage is accurately measured by crop yields. From the average yields obtained it may be safely assumed that under the soil and climatic conditions obtaining in the region where the experiments were conducted a combination of factors favorable to deep tillage does not occur often enough nor result in increases great enough to warrant its general practice.

It is mistaking or failing to recognize the purpose of plowing that leads to the belief that its efficiency increases with its depth even though that depth be extended below all practical limits of cost and effort. Plowing does not increase the water-holding capacity of the soil, nor the area in which roots may develop or from which the plants may obtain food. Plowing removes from the surface either green or dry material that may encumber it, provides a surface in which planting implements may cover the seed, and removes or delays the competition of weeds or plants other than those intended to grow, and in some cases by loosening and roughening the immediate surface checks the run-off of rain water. All these objects are accomplished as well by plowing to ordinary depths as by subsoiling, dynamiting, or deep tilling by any other method. There is little basis, therefore, for the expectation of increased yields from these practices, and the results of the experiments show that they have been generally ineffective.

EXPERIMENTS WITH SUBSOILING IN THE GREAT PLAINS

There are here reported results of subsoiling at 12 stations of this office in the Great Plains area for a total of 66 station-years, or an average of 5½ years at each station. From four to seven crops have been grown each year at each station. The crops under trial have been spring wheat, winter wheat (*Triticum aestivum*), oats (*Avena sativa*), barley (*Hordeum* spp.), flax (*Linum usitatissimum*), corn (*Zea mays*), kafir, milo, broom corn, sorghum (*Audropogon sorghum*), and cotton (*Gossypium* spp.), as shown in the results from the individual stations.

The length of time covered and the wide range of climatic conditions encountered in these experiments make the results representative of the widest range of conditions likely to be experienced in the region.

Figure 1 is a map of the Great Plains, showing the location of the field stations at which experiments have been conducted.

METHOD OF WORK

The results with subsoiling are all from continuous cropping of land to the crop under study. In this series of continuously cropped plots there are in general five methods under trial: Spring plowing, fall plowing, alternate cropping and summer tilling, subsoiling, and listing. In this study the results from the subsoiled plots, which are designated in the fields and notes as the "E plots," are compared with those from the fall-plowed plots, known as the "B plots." Except for the subsoiling practiced on E, these plots are treated exactly the same. They are plowed as early in the fall as is practicable after the crop has been removed. The plots are plowed to a good depth, the standard being set at 8 inches.

In addition to the plowing of plot E, a subsoiler is run in the bottom of the furrow, loosening the soil to a further depth of 6 to 8 inches,

or to a total depth of 14 to 16 inches. The variation from this depth is hardly more than 2 inches either way. In general, subsoiling is done for two years in succession, and then is omitted for two years. The present outline calls for subsoiling at all stations in the fall of 1915, 1918, and 1919. The ground may be worked down in the fall or left rough over the winter. In the early years of the work these plots were harrowed immediately after plowing and kept cultivated during the fall, but the tendency has been more and more to leave them rough over the winter. This is considered the better practice both for catching snow and preventing run-off and soil blowing, and at the same time it reduces the expense of crop production.

In some cases where it has been impossible to plow as early as desired, the stubble has been disked and the plowing done later.

In the spring the ground is finally prepared for seeding by the necessary use of the disk or smoothing harrow, or both.

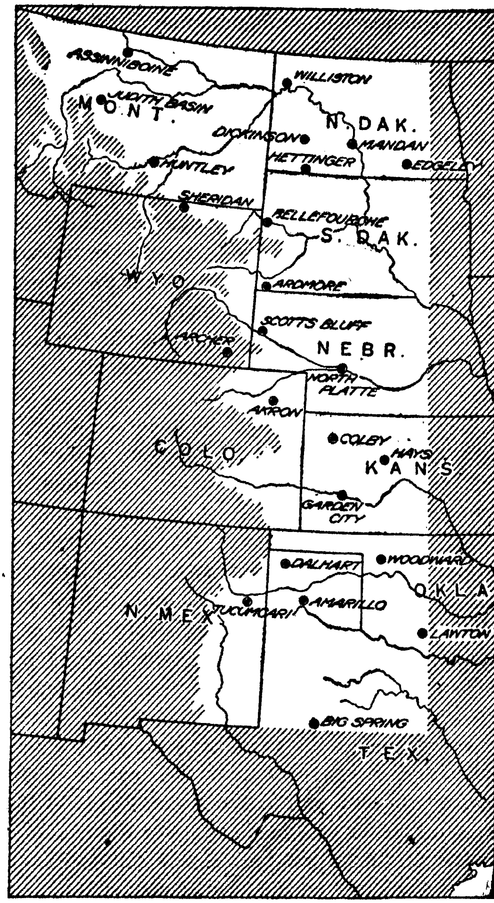

FIG. 1.—Map of the Great Plains area, which includes parts of 10 States and consists of about 400,000 square miles of territory. Its western boundary is indicated by a 5,000-foot contour. The location of each field station within the area is shown by a dot within a circle (⊙).

Seeding is done with standard farm machinery at what is believed to be the best date and rate per acre.

The plots are 2 by 8 rods, or 0.1 acre in size. The B and E plots of any one crop are separated on their long dimension by the C and D plots, or an interval of 78 feet. The different crops may be more widely separated, but all are within a field of approximately 20 acres at each station.

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METHOD OF STUDY AND PRESENTATION OF RESULTS

Tables I to XII, inclusive, present in a separate table for each field station the yields of each crop each year on plot B (not subsoiled) and on plot E (subsoiled). The average yield of each plot for the entire period of years is also presented. The principal comparisons have been made and the conclusions of the effects of subsoiling drawn from the annual and average yields. But in order to obtain a uniform expression of difference on which to calculate the probable error of the results, and through which to compare the relative effect upon different crops and at the several stations, it has seemed necessary to express the differences in some form of ratio or percentage.

A thorough study of the data has been made by means of percentages calculated on four different bases. The ratios of the average yields have been computed for each crop at each station by dividing the average yield of plot E by the average yield of plot B. This single calculation does not afford either an expression of the results each year or opportunity for the determination of the probable error based on annual differences.

In each experimental field there are a large number of plots of each crop grown each year by various methods. The difference in yield between B and E each year has been calculated as a percentage of the average yield of all plots of the same crop in the field for that year. Objection has been made to the use of this method for this particular study, for the reason that with some crops the yields of these methods are below the general average and with other crops they are above it. This results in some cases in a disproportionate valuation of the entity under study. The average departure of the results as calculated by this method from those obtained by the first method is so high as to make its use for the present study unsatisfactory.

The difference in yield between plots B and E each year has been calculated as a percentage of the yield of B, which may be considered as the control plot, or the one giving the yield that it is sought to increase on E by means of subsoiling. For the results in hand this method gives undue weight to comparatively small differences in yield as the yield of the plot selected as the base of comparison approaches zero. There are so many of these cases that the results of comparison by this method are not satisfactory when compared with actual differences in average yield.

This objectionable weighting of small differences in yield is largely overcome and the results smoothed by using the arithmetic mean of the yield of the two plots under study as the base on which to calculate the difference between the two as a percentage. This method tends, however, to reduce the percentage when it is above and to increase it when it is below 100. In over half the comparisons the ratios depart from 100 by less than 10, and within this range the distortion is not great. The average results are further made up of varying combinations of increases

and decreases, so this method is more satisfactory in the averages as determined by it than might at first be thought possible. It is this method that has been adopted for presentation. With each pair of comparable yields in Tables I to XII is given the percentage of the yield of plot E to the mean of the yield of B and E. These percentages are averaged at the right, and the probable error of this average is given. In Table XIII these average ratios and probable errors are assembled and further averaged by crops and by stations. The data in this table are presented graphically in figures 2 and 3.

In these tables and charts a percentage of 100 shows that there was no difference in the yield of the two plots, a percentage above 100 shows that the higher yield was from the subsoiled plot, and a percentage below 100 shows that the higher yield was produced on the plot not subsoiled.

It is recognized that the use of ratios weights the results, and that averages of such ratios are not the same as the ratios of the averages. They are not, therefore, accurate quantitative expressions of the results and are not a measure of the economic value of a method as compared with a control. They are not, however, in the present study misleading in direction, and serve a useful purpose of facilitating comparisons between things otherwise difficult of direct comparison.

JUDITH BASIN FIELD STATION

The field station at Moccasin, Mont., in the Judith Basin, is located on a heavy clay soil of limestone origin. The soil is apparently very rich in available fertility. It is underlain at a depth of approximately 3 feet with a limestone gravel that is closely cemented with lime materials. The gravel subsoil, which extends to a depth of about 30 feet, is practically free from soil. While it is so closely cemented that it does not unduly drain the soil, it is not of a character to allow the storage of available water or the development of roots within it.

Comparative results of fall plowing and of subsoiling are available for spring wheat, winter wheat, oats, barley, corn, and flax for the seven years 1910 to 1916, inclusive. Crops were raised on this land in 1908 and 1909, but the first subsoiling was not done until the fall of 1909. Hail in 1912 destroyed all crops except winter wheat and flax. The yields of these crops were considerably reduced by the storm, but as the damage was relatively the same on each plot, the yields are used. The winter wheat crop of 1916 was lost by winterkilling.

The E plot for each of the crops was subsoiled in the fall of 1909, 1910, 1911, 1913, and 1915.

The yields and the ratios as previously described are presented in Table I. None of the average differences observed are significant, the departures from the mean being very small and either less or only slightly greater than their probable errors. This is true of all crops except barley, which, from the results at this station alone, would appear to be

peculiarly benefited by subsoiling. This is a point not supported by the evidence of the 10 other stations at which barley is grown and therefore must be attributed to a soil difference which has been observed and to damage by gophers to the B plot in at least two seasons. While this damage has been noted in the field, it has not been taken into account in the tables. The year of 1913 is the only year in the series when all crops yielded as much, or more, on the subsoiled plot as they did on the ordinary plowed one. While it is probable that the relation is only a coincidence, it is worthy of note that no subsoiling was done in the fall of 1912 in preparation for this crop. The average results of all crops, including barley, at this station is only 103, with a probable error of 1. The conclusion is that subsoiling has been without practical effect.

TABLE I.—*Yields at the Judith Basin Field Station, Moccasin, Mont., of spring wheat, winter wheat, oats, barley, corn, and flax each year from 1910 to 1916, inclusive, on plot E, subsoiled, and plot B, not subsoiled but otherwise similarly treated, together with the average of each method for the entire period of years; the ratio of the yield on E to the mean of the yield on B and E each year; the mean ratio; and the probable error of the mean ratio*

[Yields of corn are expressed in pounds of stover per acre; of other crops in bushels of grain per acre]

Crop and plot.	Yield or ratio.								Probable error of mean ratio.
	1910	1911	1912	1913	1914	1915	1916	Average.	
Spring wheat:									
Plot B	14.0	22.0	(a)	18.5	15.8	27.0	21.1	19.7	......
Plot E	15.0	23.5	(a)	22.8	16.5	25.5	20.0	20.6	......
Ratio of E to mean	103	103	......	110	102	97	97	102	±1.3
Winter wheat:									
Plot B	24.0	23.5	12.2	23.2	16.3	28.5	0.0	18.2	......
Plot E	23.2	22.4	12.5	26.2	15.5	28.3	0.0	18.3	......
Ratio of E to mean	98	98	101	106	97	100	......	100	±0.9
Oats:									
Plot B	20.9	51.5	(a)	65.0	49.3	50.6	45.6	47.2	......
Plot E	25.3	53.0	(a)	65.0	40.6	57.1	43.1	47.4	......
Ratio of E to mean	110	101	......	100	90	106	97	101	±1.9
Barley:									
Plot B	12.5	24.1	(a)	21.9	18.1	24.0	20.5	20.2	......
Plot E	15.0	32.6	(a)	32.9	23.5	25.8	20.5	25.1	......
Ratio of E to mean	109	115	......	120	113	104	100	110	±2.2
Corn:									
Plot B	2,900	7,000	(a)	4,000	3,700	8,450	6,200	5,375	......
Plot E	3,650	4,780	(a)	5,800	5,000	8,000	6,200	5,572	......
Ratio of E to mean	111	81	......	118	115	97	100	104	±4.2
Flax:									
Plot B	6.2	13.2	5.4	12.9	9.1	16.0	10.3	10.4	......
Plot E	6.5	14.1	4.9	13.2	10.7	17.3	10.3	11.0	......
Ratio of E to mean	102	103	95	101	108	104	100	102	±0.9

[a] Crop destroyed by hail.

HUNTLEY FIELD STATION

The field station at Huntley, Mont., is located in the valley of the Yellowstone River at the foot of the first bench. The soil is a heavy gumbo clay to the depth of about 8 feet. Underlying the soil is a considerable depth of free-drained gravel.

Table II presents the yields and ratios of four years for spring wheat, oats, flax, and corn, and three years for winter wheat at this station. The only consistent results to be noted either by years or by crops are that the corn and winter-wheat crops each year have been the heavier on the subsoiled plot, while with flax the reverse has been true. The differences, however, are not enough greater than the probable error to make them significant, particularly when considered in connection with similar comparisons of the same crops at other stations.

TABLE II.—*Yields at the Huntley (Mont.) Field Station of spring wheat, winter wheat, oats, corn, and flax each year from 1913 to 1916, inclusive, on plot E, subsoiled, and plot B, not subsoiled but otherwise similarly treated, together with the average of each method for the entire period of years; the ratio of the yield on E to the mean of the yield on B and E each year; the mean ratio; and the probable error of the mean ratio*

[Yields of all crops in bushels per acre]

Croop and plot.	Yield or ratio.					
	1913	1914	1915	1916	Average.	Probable error of mean ratio.
Spring wheat:						
Plot B	11.8	20.2	25.3	7.8	16.3	
Plot E	14.5	17.5	25.5	6.2	15.9	
Ratio of E to mean	110	93	100	89	98	±3.4
Winter wheat:						
Plot B	....	25.7	13.3	12.7	17.2	
Plot E	....	27.8	13.6	15.5	19.0	
Ratio of E to mean	....	104	101	110	105	±2.0
Oats:						
Plot B	34.0	48.4	56.9	17.8	39.3	
Plot E	39.3	52.8	61.9	16.9	42.7	
Ratio of E to mean	107	104	104	97	103	±1.5
Corn:						
Plot B	14.8	13.2	40.6	25.4	23.5	
Plot E	25.7	13.9	42.5	29.6	27.9	
Ratio of E to mean	127	103	102	108	110	±4.1
Flax:						
Plot B	12.5	8.4	16.7	5.4	10.8	
Plot E	11.9	4.7	13.4	4.5	8.6	
Ratio of E to mean	98	72	89	91	88	±3.7

MANDAN FIELD STATION

The record for the Mandan (N. Dak.) Field Station covers only two years, but spring wheat has been replicated four times each year and all other crops, except flax, three times each year. On the main field the soil is a light, sandy loam with a more sandy subsoil. On this field spring wheat appears twice and the other crops once. The soil of the south field is a heavy clay loam with a heavier subsoil. All crops except flax are grown in duplicate on the south field.

TABLE III.—*Yields at the Mandan (N. Dak.) Field Station of spring wheat, oats, barley, corn, and flax for 1915 and 1916 on plot E, subsoiled, and plot B, not subsoiled but otherwise similarly treated, together with the average of each method for the two years; the ratio of the yield on E to the mean of the yield on B and E each year and replication, the mean ratio, and the probable error of the mean ratio*

[Yields in bushels per acre. Flax one, spring wheat four, and other crops three replications each year]

Crop and plot.	Yield or ratio.									
	Main field.		South field.				Main field.		Average.	Probable error of mean ratio.
			II		IV					
	1915	1916	1915	1916	1915	1916	1915	1916		
Spring wheat:										
Plot B.....	32.1	18.5	30.4	17.8	31.3	17.0	21.8	18.7	23.5	
Plot E.....	31.7	18.8	24.8	15.5	25.6	16.0	27.1	18.2	22.2	
Ratio of E to mean..	99	101	90	93	90	97	111	99	98	±1.6
Oats:										
Plot B.....	59.8	57.5	81.3	63.4	74.7	52.2	......	......	64.9	
Plot E.....	57.5	57.2	68.4	59.1	69.1	60.3	......	......	61.9	
Ratio of E to mean..	98	100	91	96	96	107	......	......	98	±1.3
Barley:										
Plot B.....	57.0	26.7	58.3	28.5	52.7	24.0	......	......	41.2	
Plot E.....	49.7	29.2	50.8	26.5	54.3	27.3	......	......	39.6	
Ratio of E to mean..	93	104	93	96	101	109	......	......	99	±2.0
Corn:										
Plot B.....	26.7	49.1	28.6	28.9	29.2	35.0	......	......	32.9	
Plot E.....	24.8	44.5	20.0	33.8	26.3	34.1	......	......	30.6	
Ratio of E to mean..	96	95	82	108	95	99	......	......	96	±2.0
Flax:										
Plot B.....	13.1	5.2	......	......	......	......	......	......	9.2	
Plot E.....	14.8	6.9	......	......	......	......	......	......	10.9	
Ratio of E to mean..	106	114	......	......	......	......	......	......	110	±3.4

The results are presented in detail in Table III. Of the 28 comparisons afforded, only 9 appear to be in favor of subsoiling. These are not confined to particular crops, to either year, or to either type of soil.

Production from all methods was heavy in both years. The mean ratio of all crops is 100, with a probable error of 1.6. With all crops except flax the average result is slightly against subsoiling, but by a margin less than the calculated probable error. Flax from an unduplicated pair of plots shows an apparent increase from subsoiling, but as this result with this crop in comparison with others is exactly contrary to those obtained at Huntley it must be concluded that the departures in each case are due to the experimental error. If this conclusion is correct, subsoiling has been without significant effect at this station.

BELLEFOURCHE FIELD STATION

The Bellefourche Field Station, near Newell, S. Dak., is located on a heavy clay soil derived from the decomposition of Pierre shale. From the soil at the surface there is a rapid change to broken but undecomposed shale. Near the bottom of the second foot there is a comparatively impervious layer of soil.

TABLE IV.—*Yields at the Bellefourche (S. Dak.) Field Station of spring wheat, winter wheat, oats, corn, and barley each year from 1909 to 1916, inclusive, on plot E, subsoiled, and plot B, not subsoiled but otherwise similarly treated, together with the average of each method for the entire period of years; the ratio of the yield on E to the mean of the yield on B and E each year; the mean ratio; and the probable error of the mean ratio*

[Yields of all crops in bushels per acre]

Crop and plot.	Yield or ratio.									
	1909	1910	1911	1912	1913	1914	1915	1916	Average.	Probable error of mean ratio.
Spring wheat:										
Plot B	23.3	0.0	0.0	0.0	7.9	4.8	57.7	10.8	13.1	
Plot E	28.5	0.0	0.0	0.0	6.8	4.7	55.6	16.6	14.0	
Ratio of E to mean	110	......	......	......	93	99	98	121	104	± 3.8
Winter wheat:										
Plot B	34.4	0.0	0.0	0.0	21.8	13.4	20.4	8.8	12.4	
Plot E	29.3	0.0	0.0	0.0	18.7	14.7	25.2	8.5	12.1	
Ratio of E to mean	92	......	......	......	92	105	111	98	100	± 2.9
Oats:										
Plot B	46.9	0.0	0.0	6.6	15.8	24.7	119.7	23.1	29.6	
Plot E	60.8	0.0	0.0	7.3	16.3	20.3	118.1	21.1	30.5	
Ratio of E to mean	113	......	......	105	102	90	99	95	101	± 2.3
Barley:										
Plot B	25.0	4.8	0.0	0.0	8.9	7.1	71.5	33.6	18.9	
Plot E	33.8	0.0	0.0	0.0	7.8	6.3	78.9	29.2	19.5	
Ratio of E to mean	115	0.0	......	......	93	94	105	93	83	±10.6
Corn:										
Plot B	23.5	0.0	0.0	29.7	6.5	0.0	53.0	31.2	18.0	
Plot E	20.8	0.0	0.0	26.3	9.4	0.0	47.7	32.3	17.1	
Ratio of E to mean	94	......	......	94	118	......	95	102	101	± 3.2

Table IV presents eight years' results with spring wheat, winter wheat, oats, barley, and corn. During these years conditions have ranged from the drouth of 1911, which was so severe that all crops on all methods of preparation were total failures, to the favorable conditions of 1915, when the highest yields of spring grains yet recorded in experimental work in the Great Plains were obtained.

Of the 40 comparisons offered, only 11 are in favor of subsoiling. These are so evenly distributed throughout the different crops and years that no positive conclusion can be derived from them. A negative conclusion that subsoiling has afforded no protection against drouth is very clearly indicated. In the average of the eight years the differences in yield as a result of the two methods are measured in fractions of a bushel with every crop. The departures of the average ratios from 100 are all less than the probable error except in the case of barley, where the departure is less than twice the probable error. With this crop the difference in average number of bushels per acre is in one direction, while the departure of the average ratios is in the other. This is partly explained by the fact that in 1910 there was a production of nearly 5 bushels per acre on the fall plowing, while the subsoiled plot was a total failure. The mean ratio of all crops is 98, with a probable error of 2.5 This shows practically no effect in either direction as an average result of subsoiling.

ARDMORE FIELD STATION

The soil on the Ardmore (S. Dak.) Field Station is a heavy clay loam with a lighter subsoil. The subsoil is not uniform, but at depths of 3 feet or more it generally breaks into sand or gravel.

Three years' results, exclusive of the year 1914, when the crop was completely destroyed by hail, are available for study. One year the crops from many methods, including the two under study, were total failures. One year the yields were good, and one year the yields were very high.

Table V presents in detail the results with spring wheat, winter wheat, oats, barley, and corn at this station. With all crops except winter wheat, with which the better yield both years has been from subsoiling, the evidence is all against that practice. It failed with all crops to overcome the drouth of 1913, and actually appeared to reduce the yields of all crops but winter wheat both in the exceptionally productive year of 1915 and in the more normal year of 1916. The average ratio of all crops is 93, with a probable error of 3.1. There apparently is at this station a detrimental effect from subsoiling. The decrease in yield is not, however, enough greater than its probable error to make it all certain that it might not be effaced by continuation of the record.

TABLE V.—*Yields at the Ardmore (S. Dak.) Field Station of spring wheat, winter wheat, oats, barley, and corn each year from 1913 to 1916, inclusive, on plot E, subsoiled, and plot B, not subsoiled but otherwise similarly treated, together with the average of each method for the entire period of years; the ratio of the yield on E to the mean of the yield on B and E each year; the mean ratio; and the probable error of the mean ratio*

[Yields of all crops in bushels per acre]

Crop and plot.	Yield or ratio.					
	1913	1914	1915	1916	Average.	Probable error of mean ratio.
Spring wheat:						
Plot B	0.0	(a)	49.5	17.5	22.3	
Plot E	0.0	(a)	43.3	12.8	18.7	
Ratio of E to mean	....	....	93	84	89	±3.8
Winter wheat:						
Plot B	0.0	(a)	29.2	29.8	19.7	
Plot E	0.0	(a)	33.3	34.5	22.6	
Ratio of E to mean	....	....	107	107	107	± .0
Oats:						
Plot B	0.0	(a)	75.4	42.2	39.2	
Plot E	0.0	(a)	59.4	25.0	28.1	
Ratio of E to mean	....	....	88	74	81	±5.9
Barley:						
Plot B	0.0	(a)	54.0	25.2	26.4	
Plot E	0.0	(a)	51.0	24.7	25.2	
Ratio of E to mean	....	....	97	99	98	±0.8
Corn:						
Plot B	0.0	0.0	43.7	28.7	18.1	
Plot E	0.0	0.0	32.9	26.6	14.9	
Ratio of E to mean	....	....	86	96	91	±4.2

[a] Crop destroyed by hail.

SCOTTSBLUFF FIELD STATION

The soil of the Scottsbluff (Nebr.) Field Station is a comparatively light sandy loam. At a depth varying from 5 to 8 feet there is a sharp break from this soil to either sand or Brule clay. Above this point the soil offers no unusual resistance either to the downward passage of water or to the development of roots.

Table VI presents five years' results with spring wheat, oats, barley, and corn at this station. Considered either by crops or by years, the only consistency to be noted is the heavier production on the subsoil plots in 1912. In the average of the five years the differences exhibited are very small and are less than the probable error with all crops except barley, where the difference only slightly exceeds the probable error.

TABLE VI.—*Yields at the Scottsbluff (Nebr.) Field Station of spring wheat, oats, barley, and corn each year from 1912 to 1916, inclusive, on plot E, subsoiled, and plot B, not subsoiled but otherwise similarly treated, together with the average of each method for the entire period of years; the ratio of the yield on E to the mean of the yield of B and E each year; the mean ratio; and the probable error of the mean ratio*

[Yields of all crops in bushels per acre]

Crop and plot.	Yield or ratio.						
	1912	1913	1914	1915	1916	Average.	Probable error of mean ratio.
Spring wheat:							
Plot B	6.3	7.8	6.7	16.2	5.7	8.5	
Plot E	12.3	6.3	9.5	6.8	7.0	8.4	
Ratio of E to mean	132	89	117	59	110	101	±9.3
Oats:							
Plot B	21.6	16.9	14.7	39.7	7.2	20.0	
Plot E	27.8	17.5	15.9	48.1	2.8	22.4	
Ratio of E to mean	113	102	104	110	56	97	±6.9
Barley:							
Plot B	23.5	....	4.4	35.4	10.0	18.3	
Plot E	24.8	....	5.2	26.0	6.0	15.5	
Ratio of E to mean	103	....	108	85	75	93	±6.2
Corn:							
Plot B	38.0	32.2	20.1	10.1	22.1	24.5	
Plot E	40.0	26.1	14.2	13.6	23.2	23.4	
Ratio of E to mean	103	90	83	115	102	99	±4.1

ARCHER FIELD STATION

The soil of the field station at Archer, Wyo., is a medium-dark sandy loam with a little fine gravel distributed very evenly through it. Below the second foot the proportion of sand increases, and at a depth varying from 3 to 6 feet pure gravel is reached.

Table VII presents three years' results with spring wheat, winter wheat, and oats, and two years' results with corn and barley at this station. Of the 13 comparisons afforded only 5 appear to be in favor of subsoiling. Only with corn has the heavier yield for more than one year been from the subsoiled plot. The average differences exhibited by all crops are insignificant when considered in connection with the probable error.

TABLE VII.—*Yields at the Archer (Wyo.) Field Station of spring wheat, winter wheat, oats, barley, and corn each year from 1914 to 1916, inclusive, on plot E, subsoiled, and plot B, not subsoiled but otherwise similarly treated, together with the average of each method for the entire period of years; the ratio of the yield on E to the mean of the yield on B and E each year; the mean ratio; and the probable error of the mean ratio*

[Yield of corn in pounds of stover; other crops in bushels of grain per acre]

Crop and plot.	Yield or ratio.				
	1914	1915	1916	Average.	Probable error of mean ratio.
Spring wheat:					
Plot B	7.5	23.7	2.4	11.2	
Plot E	5.8	25.0	1.0	10.6	
Ratio of E to mean	87	103	59	83	± 9.6
Winter wheat:					
Plot B	0.0	24.7	7.4	10.7	
Plot E	0.0	24.2	2.4	8.9	
Ratio of E to mean	....	99	49	74	±21.1
Oats:					
Plot B	14.5	35.9	3.7	18.0	
Plot E	9.4	34.7	5.6	16.6	
Ratio of E to mean	79	98	120	99	± 8.4
Barley:					
Plot B	....	29.8	5.2	17.5	
Plot E	....	35.8	4.2	20.0	
Ratio of E to mean	....	109	89	99	± 8.5
Corn:					
Plot B	1,090	3,900	([a])	2,495	
Plot E	1,130	4,450	([a])	2,790	
Ratio of E to mean	102	107	....	105	± 2.1

[a] Weights lost.

AKRON FIELD STATION

The soil of the field station at Akron, Colo., is a clay loam locally known as "hard land." It is characterized in the native vegetation by a growth of short grass.

Table VIII presents an unbroken record of eight years' results with spring wheat, oats, barley, and corn, and seven years' results with winter wheat at this station. The E plots were subsoiled in the fall of 1908, 1909, 1812, 1913, and 1914. Of the 39 comparisons presented in this table only 7 show the higher yields from the subsoiled plot. The only consistency in the distribution of these among the different crops or years is that in 1909 all four crops under trial that year showed the heavier yield on the subsoiled plot. In the average of the eight years the better yield of each crop has been obtained from the plot not subsoiled. The average decrease in yield as a result of subsoiling ranges from 1.7 bushels per acre with winter wheat to 3.4 bushels per acre with corn. The

average ratio for all crops is 85. This station shows the clearest cut and most decisive results of any station where subsoiling has been investigated.

Table VIII.—*Yields at the Akron (Colo.) Field Station of spring wheat, winter wheat, oats, barley, and corn each year from 1909 to 1916, inclusive, on plot E, subsoiled, and plot B, not subsoiled but otherwise similarly treated, together with the average of each method for the entire period of years; the ratio of the yield on E to the mean of the yield on B and E each year; the mean ratio; and the probable error of the mean ratio*

[Yields of all crops in bushels per acre]

Crop and plot.	Yield or ratio.									
	1909	1910	1911	1912	1913	1914	1915	1916	Average.	Probable error of mean ratio.
Spring wheat:										
Plot B	10.3	6.2	4.1	20.7	0.8	12.2	25.8	3.8	10.5	
Plot E	11.2	5.5	1.5	16.0	.5	9.8	23.7	1.7	8.7	
Ratio of E to mean	104	94	54	87	77	89	96	62	83	±4.4
Winter wheat:										
Plot B	......	10.3	6.8	26.7	2.0	24.8	20.8	4.2	13.7	
Plot E	......	6.9	3.3	21.2	3.2	24.5	21.0	3.8	12.0	
Ratio of E to mean	......	80	65	89	123	99	100	95	93	±4.4
Oats:										
Plot B	14.1	8.0	15.9	46.9	.6	36.9	57.2	7.2	23.4	
Plot E	16.1	11.3	8.4	35.3	0.0	30.3	57.5	4.1	20.4	
Ratio of E to mean	107	117	69	86	0.0	90	100	73	80	±7.9
Barley:										
Plot B	16.8	10.5	16.3	27.9	3.1	36.7	47.9	5.0	20.5	
Plot E	19.8	6.9	5.2	22.5	1.5	27.9	52.3	4.2	17.5	
Ratio of E to mean	108	79	48	89	65	86	104	91	84	±4.7
Corn:										
Plot B	27.3	18.3	0.0	46.9	9.9	17.3	29.2	4.8	19.2	
Plot E	32.8	12.7	0.0	37.1	4.3	13.9	22.3	3.2	15.8	
Ratio of E to mean	109	82	......	88	61	89	87	80	85	±3.2

HAYS FIELD STATION

The soil of the field station on which the experimental work has been conducted at Hays, Kans., is a heavy silt loam. Penetration of water to the lower depths is slow, the very compact soil in the third foot offering marked resistance to its downward passage.

Table IX presents from this station a record beginning with 1907 for winter wheat and corn; 1908 for spring wheat, oats, and barley; 1911 for kafir; and 1912 for milo. In the average of all the years the higher yields are from the subsoiled plots with all crops except barley, which shows no difference. With corn the higher yield has been obtained

every year from the subsoiled plot. Winter wheat, corn, and kafir show increases amounting to more than three times the probable error. With the other crops the differences are less than the probable error. This is the only station at which the averages of all crops show a ratio of 100 or more on the subsoiled plot. With some of the crops the average yields have been so small as to be unprofitable from either method. It should be noted that this station has the highest annual precipitation of any station under study. As noted later in discussing the use of dynamite and deep plowing, the results of those practices do not support those obtained from subsoiling.

TABLE IX.—*Yields at the Hays (Kans.) Field Station of spring wheat, winter wheat, oats, barley, corn, kafir, and milo each year from 1907 to 1916, inclusive, on plot E, subsoiled, and plot B, not subsoiled but otherwise similarly treated, together with the average of each method for the entire period of years; the ratio of the yield on E to the mean of the yield on B and E each year; the mean ratio; and the probable error of the mean ratio*

[Yields of all crops in bushels per acre]

Crop and plot.	Yield or ratio.											
	1907	1908	1909	1910	1911	1912	1913	1914	1915	1916	Average.	Probable error of mean ratio.
Spring wheat:												
Plot B	......	4.5	(a)	9.6	0.0	15.2	0.5	(b)	7.0	0.7	5.4	
Plot E	......	5.2	(a)	12.6	0.0	12.7	1.3	(b)	8.3	.3	5.8	
Ratio of E to mean	......	107	......	114	......	91	144	......	108	60	104	±7.2
Winter wheat:												
Plot B	18.2	23.2	(a)	27.8	.3	13.8	2.3	24.8	13.1	22.7	16.2	
Plot E	13.6	30.5	(a)	39.8	.3	20.1	4.1	25.3	14.9	27.6	19.6	
Ratio of E to mean	86	114	......	118	100	119	128	101	106	110	109	±2.9
Oats:												
Plot B	......	3.7	(a)	16.6	0.0	37.7	10.6	27.0	25.1	6.0	15.8	
Plot E	......	17.9	(a)	24.5	0.0	45.1	21.8	26.6	29.2	1.0	20.8	
Ratio of E to mean	......	166	......	119	......	109	135	99	108	29	109	±9.1
Barley:												
Plot B	......	12.4	(a)	19.7	0.0	28.8	4.0	16.7	26.8	10.1	14.8	
Plot E	......	14.8	(a)	19.3	0.0	33.8	4.6	15.2	22.4	8.2	14.8	
Ratio of E to mean	......	109	......	99	......	108	107	95	91	90	100	±2.4
Corn:												
Plot B	12.2	3.1	7.9	6.8	(c)	1.8	(c)	5.5	14.9	3.6	7.0	
Plot E	13.6	8.8	8.0	7.4	(c)	5.5	(c)	6.4	16.5	4.3	8.8	
Ratio of E to mean	105	148	101	104	......	151	......	108	105	109	116	±5.2
Kafir:												
Plot B	......	......	......	......	.6	12.8	(c)	16.3	67.4	.4	19.5	
Plot E	......	......	......	......	1.0	26.1	(c)	23.0	66.6	.7	23.5	
Ratio of E to mean	......	......	......	......	125	134	......	117	99	127	120	±4.2
Milo:												
Plot B	......	......	......	......	(c)	16.2	(c)	15.2	53.5	1.4	21.6	
Plot E	......	......	......	......	(c)	17.5	(c)	17.6	48.8	1.3	21.3	
Ratio of E to mean	......	......	......	......	......	104	......	107	95	96	101	±2.4

[a] Destroyed by hail. [b] Destroyed by soil blowing. [c] Destroyed by insects.

GARDEN CITY FIELD STATION

The work at the field station at Garden City, Kans., is on a high upland. The soil is a light silt loam. With the exception of the accumulated humus near the surface it is practically uniform to a depth of at

least 15 feet. The development of roots is limited only by the depth to which water is available and the physiological character of the plant.

Table X presents the results of seven years' work at this station, exclusive of 1913, when the crops were destroyed by hail. In 1911, which is included in the averages, all small-grain crops failed from drought so extreme that it was not overcome by any method under trial. In 1914 the higher yield of all five crops under trial was on the subsoiled plot. This was the only year, however, when there was a consistent, marked difference in the results from the two methods. With none of the five crops has the average departure in either direction from the mean been greater than 4 per cent. Only in the case of wheat, which has a mean ratio of 103, with a probable error of 2.5, is the departure from the mean greater than the probable error. The results are conclusive in showing that subsoiling is without significant effect at this station.

TABLE X.—*Yields at the Garden City (Kans.) Field Station of spring wheat, winter wheat, oats, barley, and corn each year from 1909 to 1916, inclusive, on plot E, subsoiled, and plot B, not subsoiled but otherwise similarly treated, together with the average of each method for the entire period of years; the ratio of the yield on E to the mean of the yield on B and E each year; the mean ratio; and the probable error of the mean ratio*

[Yield of corn in total pounds; other crops in bushels per acre]

Crop and plot.	Yield or ratio.									
	1909	1910	1911	1912	1913	1914	1915	1916	Average.	Probable error of mean ratio.
Spring wheat:										
Plot B	3.2	5.2	0.0	6.3	(a)	4.3	12.6	0.0	4.5	
Plot E	2.9	5.2	0.0	7.7	(a)	5.3	12.0	0.0	4.7	
Ratio of E to mean	95	100	......	110	......	110	98	......	103	±2.5
Winter wheat:										
Plot B	0.0	0.0	0.0	0.0	(a)	6.3	10.0	0.0	2.3	
Plot E	0.0	0.0	0.0	0.0	(a)	6.7	9.9	0.0	2.4	
Ratio of E to mean	......	......	......	......	......	103	99	......	101	±1.7
Oats:										
Plot B	3.2	10.3	0.0	23.1	(a)	8.1	32.7	3.0	11.5	
Plot E	2.6	10.0	0.0	15.9	(a)	17.3	30.9	3.0	11.4	
Ratio of E to mean	90	99	......	82	......	136	97	100	101	±4.5
Barley:										
Plot B	4.8	5.4	0.0	9.0	(a)	15.2	24.2	3.0	8.8	
Plot E	3.7	5.2	0.0	8.5	(a)	17.3	21.5	4.0	8.6	
Ratio of E to mean	87	98	......	97	......	106	94	114	99	±2.6
Corn:										
Plot B	(b)	(b)	1,400	4,620	(a)	3,040	1,900	1,200	2,440	
Plot E	(b)	(b)	750	4,500	(a)	4,840	1,800	2,260	2,830	
Ratio of E to mean	......	......	70	99	......	123	97	131	104	±7.8

[a] Crop destroyed by hail. [b] Weights lost.

AMARILLO FIELD STATION

The soil at the field station at Amarillo, Tex., is a heavy clay silt. The storage of water and the development of the feeding roots of the crop are apparently interfered with by comparatively impervious soil in the third foot.

Eight years' results with spring wheat, winter wheat, oats, and barley, and nine years' with corn are presented in Table XI. The year 1910 is not included, as the location of the station was changed, and the preparation of the land was uniform for that year. Of the 41 comparisons afforded in this table less than one-third show the higher yield to have been from the subsoiled plot. There is no consistency in the distribution of these either by years or by crops. In the average of the entire period the results with all crops are against subsoiling. The average decrease in yields as a result of subsoiling ranges from 1 bushel per acre with spring wheat to 3.2 bushels with oats. With all crops except corn the decrease due to subsoiling is more than twice the probable error.

TABLE XI.—*Yields at the Amarillo (Tex.) Field Station of spring wheat, winter wheat, oats, barley, and corn each year from 1907 to 1916, inclusive, on plot E, subsoiled, and plot B, not subsoiled but otherwise similarly treated, together with the average of each method for the entire period of years; the ratio of the yield on E to the mean of the yield on B and E each year; the mean ratio; and the probable error of the mean ratio*

[Yield of all crops in bushels per acre]

Crop and plot.	Yield or ratio.										
	1907	1908	1909[a]	1911	1912	1913	1914	1915	1916	Average.	Probable error of mean ratio.
Spring wheat:											
Plot B	......	14.0	2.8	10.0	8.5	1.8	12.8	11.0	7.0	8.5	
Plot E	......	16.2	4.0	11.3	4.2	.8	12.3	10.3	1.2	7.5	
Ratio of E to mean	......	107	118	106	66	62	98	97	29	85	±7.9
Winter wheat:											
Plot B	......	14.3	0.0	3.5	7.2	1.3	23.0	24.4	3.6	9.7	
Plot E	......	16.5	0.0	1.2	3.3	1.3	19.3	19.7	4.5	8.2	
Ratio of E to mean	......	107	......	51	63	100	91	89	111	87	±6.1
Oats:											
Plot B	......	32.2	0.0	27.5	14.1	2.5	30.9	31.7	12.2	18.9	
Plot E	......	28.1	0.0	19.2	8.8	4.1	30.6	34.2	.9	15.7	
Ratio of E to mean	......	93	......	82	77	124	100	104	14	85	±8.0
Barley:											
Plot B	......	13.2	5.8	11.7	1.7	0.0	16.7	17.3	3.8	8.8	
Plot E	......	11.9	0.0	10.3	1.5	0.0	17.1	16.0	.2	7.1	
Ratio of E to mean	......	95	0.0	94	94	......	101	96	10	70	±12.0
Corn:											
Plot B	1.4	22.9	2.7	9.2	.7	0.0	3.6	54.0	7.2	11.3	
Plot E	1.1	25.7	1.7	7.1	1.0	0.0	5.1	46.4	3.7	10.2	
Ratio of E to mean	88	106	77	87	118	......	117	92	68	94	±4.7

[a] No record for 1910 on account of change in location of station.

TUCUMCARI FIELD STATION

The soil of the field station at Tucumcari, N. Mex., is of a residual type and is classified by the Bureau of Soils as a fine sand. The sand extends down to a depth of from 1 to 3 feet, gradually blending into a clay which continues in many places to a depth of at least 135 feet.

Table XII presents three years' results with kafir, milo, sorghum, broom corn, and cotton at this station. Of the 15 comparisons afforded in this table only 2 are in favor of subsoiling. These are kafir in 1914 and broom corn in 1915. This evidence seems conclusive that subsoiling here is at least an unnecessary if not a detrimental practice.

TABLE XII.—*Yields at the Tucumcari (N. Mex.) Field Station of kafir, milo, sorghum, broom corn, and cotton each year from 1914 to 1916, inclusive, on plot E, subsoiled, and plot B, not subsoiled but otherwise similarly treated, together with the average of each method for the entire period of years; the ratio of the yield on E to the mean of the yield on B and E each year; the mean ratio; and the probable error of the mean ratio*

[Yields of kafir and milo in bushels; sorghum in pounds of forage; broom corn in pounds of brush; and cotton in pounds of seed cotton per acre]

Crop and plot.	Yield or ratio.				
	1914	1915	1916	Average.	Probable error of mean ratio.
Kafir:					
Plot B	34.9	39.8	18.0	30.9	
Plot E	38.5	39.8	18.0	32.1	
Ratio of E to mean	105	100	100	102	±1.4
Milo:					
Plot B	45.8	46.8	9.0	33.9	
Plot E	36.2	41.1	7.2	28.2	
Ratio of E to mean	88	94	89	90	±1.4
Sorghum:					
Plot B	5,320	5,020	5,560	5,300	
Plot E	5,080	4,980	4,600	4,887	
Ratio of E to mean	98	100	91	96	±2.2
Broom corn:					
Plot B	583	500	420	501	
Plot E	490	780	190	487	
Ratio of E to mean	91	122	62	92	±12.2
Cotton:					
Plot B	838	520	380	579	
Plot E	773	340	205	439	
Ratio of E to mean	96	79	70	82	± 5.8

COMPARATIVE RESULTS WITH SUBSOILING DIFFERENT CROPS

The comparative results with different crops as shown in Table XIII and figures 2 and 3 scarcely warrant any conclusion that one crop is affected differently than another by subsoiling. The average ratios with

spring wheat, winter wheat, oats, and barley, which range from 94 to 97, with probable errors ranging from 2 to 2.6, certainly do not indicate any difference in the relative effect of subsoiling upon these crops. Corn

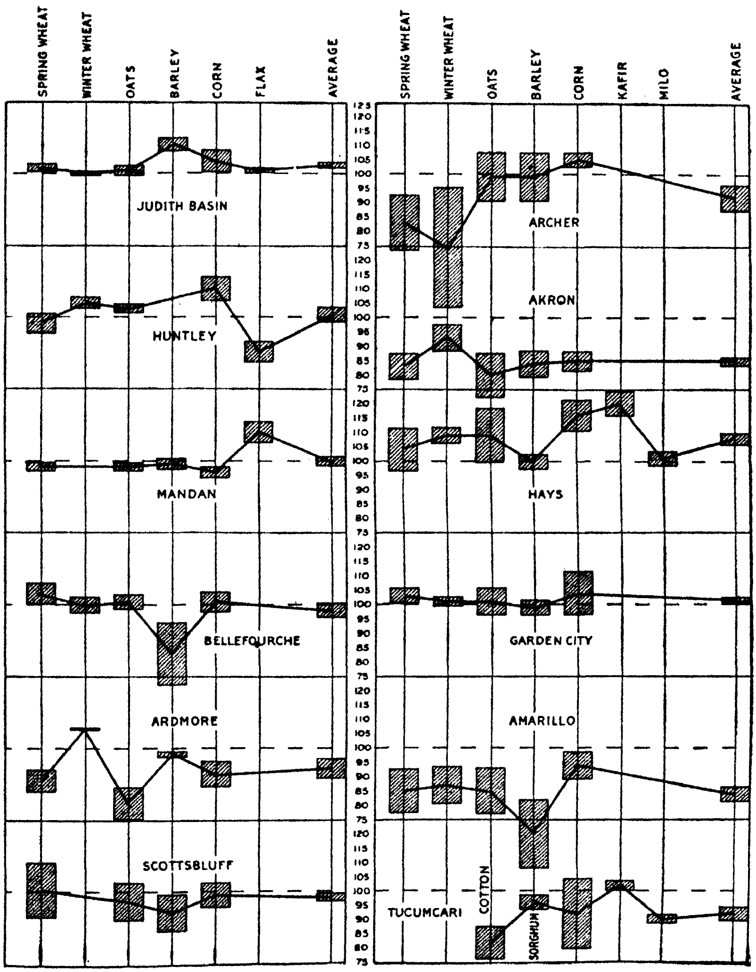

FIG. 2.—Ratio of the yield for each crop at each station of plot E (subsoiled) to the mean of the yield of plot B (not subsoiled) and plot E (subsoiled) and the averageof all crops at each station. The shaded areas are delimited by the probable error of each ratio. They mark the zones within which the chances are even that the results of a repetition of the experiments would fall.

at the same stations has a ratio of 100, with a probable error of 1.8. While the difference between this and the other crops is not great enough to make it in any way conclusive, it might perhaps indicate a slightly better response from this crop.

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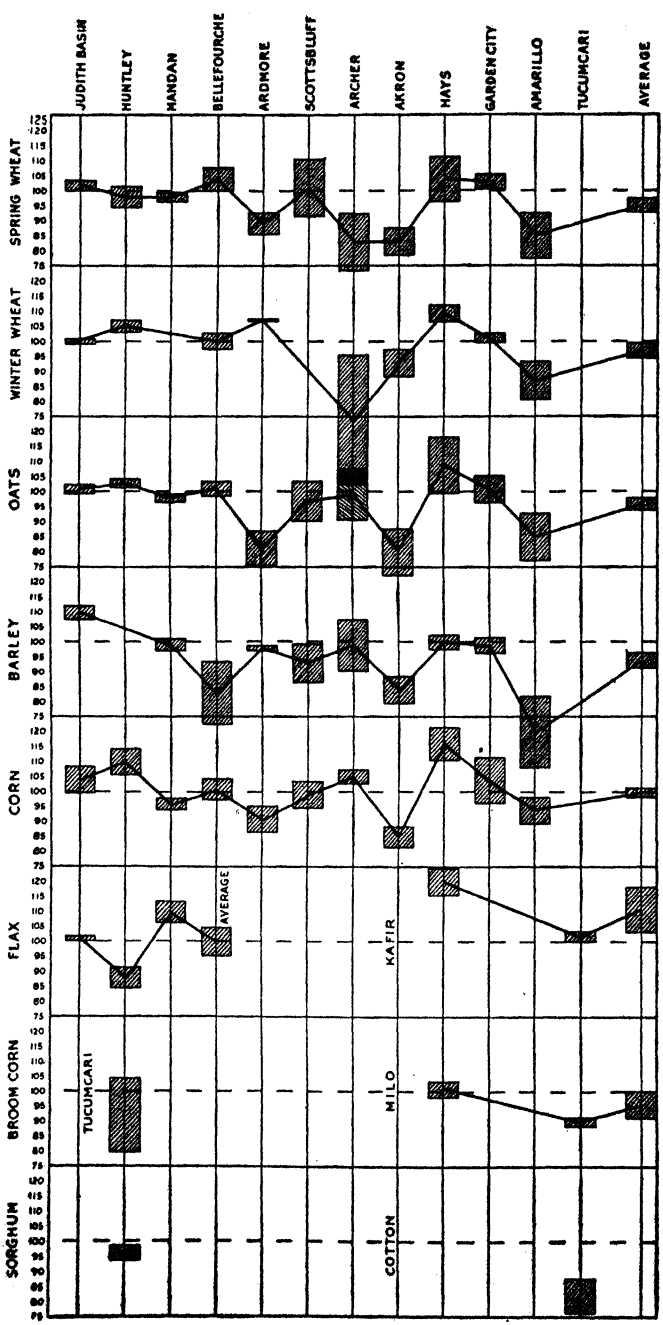

FIG. 3.—Ratio of the yield for each crop at each station of plot E (subsoiled) to the mean of the yield of plot B (not subsoiled) and plot E (subsoiled) and the average of each crop at all stations. The shaded areas are delimited by the probable errors. They mark the zones within which the chances are even that the results of a repetition of the experiments would fall.

When studied by the number of trials that resulted either in favor of or against subsoiling, it was found that, while with oats, corn, and winter wheat slightly more than half the trials resulted in favor of subsoiling and with spring wheat and barley slightly less than half, the deviations were not enough greater than their probable errors to make them significant.

The effect upon flax is apparently not different from that on the small-grain crops.

TABLE XIII.—*Summary table showing mean ratio[a] and probable error of mean of each crop at each station as shown in Tables I to XII, inclusive, and the general mean for each crop at all stations, and of all crops at each station*

Crop and factor.	Judith basin.	Huntley.	Mandan.	Belle-fourche.	Ardmore.	Scotts-bluff.	Archer.	Akron.	Hays.	Garden City.	Amarillo.	Tucum-cari.	Mean and probable error.
Spring wheat:													
Ratio	102	98	98	104	89	101	83	83	104	103	85	......	95±2.1
Probable error±	1.3	3.4	1.6	3.8	3.8	9.3	9.6	4.4	7.2	2.5	7.9	......	
Winter wheat:													
Ratio	100	105	......	100	107	......	74	93	109	101	87	......	97±2.6
Probable error±	.9	2.0	......	2.9	0.0	......	21.4	4.4	2.9	1.7	6.1	......	
Oats:													
Ratio	101	103	98	101	81	97	99	80	109	101	85	......	96±2.0
Probable error±	1.9	1.5	1.3	2.3	5.9	6.9	8.4	7.9	9.1	4.5	8.0	......	
Barley:													
Ratio	110	......	99	83	98	93	99	84	100	99	70	......	94±2.5
Probable error±	2.2	......	2.0	10.6	0.8	6.2	8.5	4.7	2.4	2.6	12.0	......	
Corn:													
Ratio	104	110	96	101	91	99	105	85	116	104	94	......	100±1.8
Probable error±	4.2	4.1	2.0	3.2	4.2	4.1	2.1	3.2	5.2	7.8	4.7	......	
Flax:													
Ratio	101	88	110	......	......	......	......	......	......	......	......	......	100±4.6
Probable error±	.9	3.7	3.4	......	......	......	......	......	......	......	......	......	
Kafir:													
Ratio	......	......	......	......	......	......	......	......	120	......	......	102	111±7.6
Probable error±	......	......	......	......	......	......	......	......	4.2	......	......	1.4	
Milo:													
Ratio	......	......	......	......	......	......	......	......	101	......	......	90	96±4.6
Probable error±	......	......	......	......	......	......	......	......	2.4	......	......	1.4	
Broom corn:													
Ratio	......	......	......	......	......	......	......	......	......	......	......	92	
Probable error±	......	......	......	......	......	......	......	......	......	......	......	12.2	
Sorghum:													
Ratio	......	......	......	......	......	......	......	......	......	......	......	96	
Probable error±	......	......	......	......	......	......	......	......	......	......	......	2.2	
Cotton:													
Ratio	......	......	......	......	......	......	......	......	......	......	......	82	
Probable error±	......	......	......	......	......	......	......	......	......	......	......	5.8	
Mean ratio	103	101	100	98	93	98	92	85	108	102	84	92	
Probable error..±	1.0	2.6	1.6	2.5	3.1	1.2	4.6	1.4	2.0	.7	2.5	2.2	

[a] Ratio based on yields of crops at the different stations as follows: Seed cotton, broom-corn brush, and sorghum forage at Tucumcari; corn stover at Judith Basin, Archer, and Garden City; ear corn at Huntley, Mandan, Bellefourche, Ardmore, Scottsbluff, Akron, Hays, and Amarillo; all other crops on yields of grain at all stations.

Kafir at the two stations from which results with this crop have been obtained appears to have given a markedly favorable response to subsoiling in comparison with the effect of that practice upon other crops, particularly milo. This subject is being given much fuller investigation at a number of additional stations to which the crop is adapted.

The results with sorghum, cotton, and broom corn being from a single station can scarcely be compared with those obtained with other crops.

The grand average ratio of all crops at all stations is 97, with a probable error of 0.9. This average, of course, is meaningless in its possible application to any crop or any station. It would be influenced by the distribution of the observations among conditions that were either favorable or unfavorable in their response. The only purpose of introducing it here is to show the relative lack of any effect, and particularly of any favorable effect, of the practice of subsoiling as applying to a wide territory and a wide range of crops.

This lack of effect of the practice when applied generally to all crops and to the entire area is further confirmed when the results are studied in another way. Exclusive of years of total failure there are 353 trials here reported. In 15 of these there was no difference in the yield from the two methods, in 153 cases the higher yield was obtained from the subsoiled plot, and in 185 cases from the plot not subsoiled.

COMPARATIVE RESULTS WITH SUBSOILING IN FAVORABLE AND UNFAVORABLE YEARS

The relative effect of subsoiling in favorable and unfavorable years is a question that naturally arises. There have been a number of cases in which the crops by both methods have been a total failure. *There have been some cases in which the plot not subsoiled produced a small crop when the subsoiled plot was a total failure. There has been no case in the history of the experiments when the reverse was true.* In order to obtain definite information on the subject the results were divided into two groups; one group containing the ratios from each station for those years when the yield was above the average for that station and the other group containing the ratios of those years when the yield was below the average. Those groups showed average ratios of 100 for the years of better production and 94.4 for the years of poorer production.

In the years above the average in production 75 trials resulted in favor of subsoiling, 81 trials in favor of ordinary plowing, and in 8 trials the same yield was obtained from each method. In the years below the average in production 78 trials resulted in favor of subsoiling, 104 in favor of ordinary plowing, and 7 showed no difference in the yields from the two methods.

These results indicate that, on the average, subsoiling, instead of overcoming the effects of drouth, actually intensifies them. In this connection it should be recognized that, while low yields are in some cases caused by fungus diseases, by insect attacks, or by unfavorable temperature or other weather conditions, the one primary predominating cause of low yields has been the lack of sufficient soil moisture at some time during the growth of the crop.

DEEP TILLING BY THE USE OF DYNAMITE OR SPECIAL PLOWS

Experiments have been conducted with both dynamite[1] and the Spalding deep-tilling machine at the Hays and Akron stations, and with dynamite at the Ardmore, Bellefourche, and Judith Basin stations.

HAYS FIELD STATION

DEEP TILLING BY THE USE OF DYNAMITE AND SPECIAL PLOWS

In 1913 a series of experiments was started at Hays, Kans., to determine the effect of different methods of preparing the land for winter wheat in a series of three-year rotations of fallow, winter wheat, and kafir. In this series four rotations, No. 501, 502, 503, and 504, are identical except as noted below. In No. 501 the plowing for the fallow is done with a Spalding deep-tilling machine, which plows the soil to a depth of from 12 to 14 inches. This plowing is done in the fall, preceding the fallow season, or practically an entire year before seeding to winter wheat. In rotation 502, dynamite is used in the fall. After dynamiting, the land is furrowed with a lister, the same as in rotations 503 and 504. In dynamiting, 18 shots of half sticks of 20 per cent powder placed 3 feet deep are fired on the tenth-acre plot, the distance between the shots being 16 feet. The plots to be fallowed in rotations 503 and 504 are furrowed out with the lister in the fall preceding the fallow season. These two rotations are identical except that the wheat stubble in No. 503 is disked after harvest, while that in No. 504 receives no cultivation until both are furrowed with a lister in the fall. All the fallow plots are given necessary cultivation to keep them free from vegetation during the fallow year. These rotations were begun in the spring of 1913, but the crops that year were a failure. The first dynamiting and deep tilling was done in the fall or 1913. The land so treated was fallow in 1914, so the first crop of wheat on plots differing in their treatment was harvested in 1915. The first kafir following the wheat on the plots differently treated was produced in 1916.

In Table XIV are given the yields of both winter wheat and kafir for the three years 1914, 1915, and 1916. There are thus shown one wheat crop, 1914, on land uniform in preparation, and two wheat crops, 1915 and 1916, on land differing in its treatment. With the kafir crop the preparation of the various rotations was uniform for the crops of 1914 and 1915, but was differentiated for the crop of 1916. To facilitate comparisons, the data in this table are shown in two forms. First, the yield in bushels, and second, the ratio of these yields to the mean yield of the four plots for each year. The data from plots differing in their treatment are shown in boldfaced type.

[1] The E. I. du Pont de Nemours Powder Co. furnished the material for the dynamiting experiments and experts to direct the operations at the different field stations.

It appears from these results that no significant differences in the yields have resulted from the differences in preparation. There are no greater differences exhibited between the deep-tilled or dynamited plots and those not so treated than are shown between the same plots when the preparation of the land was uniform, or are shown between rotations 503 and 504, which are practically the same in their treatment.

TABLE XIV.—*Yields at the Hays (Kans.) Field Station for the years 1914 to 1916, inclusive, from four 3-year rotations of fallow, winter wheat, and kafir, showing the results of dynamiting and deep tillage of fallow*

Rotation and crop.	Yield in bushels.			Ratio of yield to mean.		
	1914	1915	1916	1914	1915	1916
Rotation 501:						
Fallow, deep-tilled	......	......	......	......	......	......
Winter wheat	24.8	14.9	38.8	98	108	107
Kafir	2.6	51.2	12.7	52	104	105
Rotation 502:						
Fallow, dynamited	......	......	......	......	......	......
Winter wheat	24.3	13.5	32.9	96	98	91
Kafir	5.0	46.4	9.5	100	94	79
Rotation 503:						
Fallow, listed	......	......	......	......	......	......
Winter wheat	25.5	14.4	35.5	100	104	98
Kafir	6.4	46.8	11.4	128	93	94
Rotation 504:						
Fallow, listed	......	......	......	......	......	......
Winter wheat	26.9	12.5	37.8	106	91	104
Kafir	5.9	52.2	14.7	118	106	121

AKRON FIELD STATION

DEEP TILLAGE BY THE USE OF DYNAMITE

At the field station at Akron, Colo., a square of prairie sod was divided checkerboard fashion into 16 plots each 4 rods square, separated by the necessary alleys, making 0.1 acre in each plot. The designation of the plots by letters is similar to that at Ardmore (fig. 4). On August 26 and 27, 1912, the two center tiers of plots running north and south were dynamited. The soil was quite dry at this time. Twenty per cent dynamite was used, the shots being placed 15 feet apart, 16 holes to the plot. The shots were fired at a depth of 30 inches, ½ pound of dynamite being used for each charge. After a rain which put the soil in good condition the entire block of plots was broken, September 16 to 18, and the sod rolled flat with a roller.

In the spring of 1913 the eight plots on the north, four of which had been dynamited and four of which had not, were given the necessary disking and harrowing to make as good a seed bed as possible, and were

then seeded to durum wheat. The eight plots composing the two tiers on the south side of the block were replowed and the seed bed prepared with the disk and harrow. These eight plots were planted to corn.

The season proved very dry, and both crops were a failure on all plots. The late breaking was not considered a favorable preparation because of the lack of water in storage in the soil. Under favorable conditions of spring and summer rainfall it would have produced a crop, but under the conditions that actually obtained only failure was to be expected.

No effect of the blasting could be observed in the crop. Where a charge of dynamite had been set, there was a slight depression and the wheat in this space was an inch or two taller than that surrounding it, but no taller than it was in other depressions not caused by blasting.

The wheat plots were plowed 5 inches deep on September 23. The corn plots were plowed 5 inches deep on July 15, when the corn was so badly damaged by drought that it was evident there would be no crop produced. The same plots were replanted to wheat and corn in 1914. The yields are given in Table XV. The difference between the average yield of wheat on the four plots dynamited and on the four plots not dynamited is well within the probable error of the series. The average yield of corn on the plots not dynamited was 14.1 bushels, with a probable error of 1.5 bushels, while on the dynamited plots the average yield was 18.6 bushels, with a probable error of 1.3 bushels. Even the apparent increase in yield, which the probable error shows is open to question that it may have been due to accidental causes, is in no way commensurate with the expense of dynamiting, even if the effect persisted for a number of years.

TABLE XV.—*Yields of wheat and corn (bushels per acre) in 1914 at the Akron (Colo.) Field Station on land dynamited in 1912, and on control plots not dynamited*

Crop and treatment.	Plot and yield.				Average.	Probable error.
Wheat:						
Not dynamited	A 17.8	C 15.7	K 13.0	M 15.3	15.5	±0.6
Dynamited	B 14.4	D 16.1	J 14.0	L 15.6	15.0	±0.4
Corn:						
Not dynamited	E 17.6	G 10.4	O 11.6	Q 16.7	14.1	±1.5
Dynamited	F 13.4	H 20.0	N 18.9	P 22.2	18.6	±1.3

In 1915 the eight plots constituting the west half of the block were planted to corn and the eight plots constituting the east half to wheat. The average yield of ear corn was 33.2 bushels per acre. The average yield of the four plots dynamited in the fall of 1912 was exactly the same

as that of the four not dynamited. The individual yields of the four wheat plots following wheat were lost by mixture. There was no difference in height, stand, or estimated yield. Of the four wheat plots following corn two were dynamited in 1912. Their yield was 18.2 bushels on plot J and 19.2 bushels on plot L. Plot K, not dynamited, yielded 16.2 bushels, and plot M, not dynamited, 18.2 bushels. This shows an average gain of 1.5 bushels per acre in favor of the pair of dynamited plots, but exactly the same difference is shown between the pairs J–K and L–M that do not differ in their treatment.

The experiment was continued in 1916 by seeding the north half to corn and the south half to wheat as in 1914. The season proved unfavorable, and these plots were badly damaged by rabbits. As no differences were apparent that could be attributed to the use of the dynamite, the yields were not determined.

DEEP TILLAGE BY THE USE OF SPECIAL PLOWS

The land used in the deep-tillage experiment is a block 37 rods long north and south and 10 rods wide, divided into 16 plots 10 rods long and 2 rods wide containing ⅛ acre each. Bare, cultivated alleys 4 feet 7 inches wide separate the plots. The land was broken from prairie sod during the summer of 1907, but a record of its treatment for the seasons of 1908 and 1909 is not available. During the season of 1910 the west half of all the plots produced a light crop of oats, and the east half was planted to cultivated crops of corn, sorghum (*Andropogon sorghum*), and sunflowers (*Helianthus annuus*). The soil is a sandy loam, increasing in heaviness toward the north. The north half of the block slopes slightly to the north. The 16 plots are designated by the letters A to Q, reading from the south.

In the spring of 1911 an experiment was outlined to test the effect of deep tillage, as compared with ordinary plowing, for spring wheat and corn in different combinations of wheat and corn and the two tillage depths. Eight of the sixteen plots were to be deep-tilled and eight plowed in the ordinary manner each year; eight plots to be cropped to wheat and eight to corn in such manner as to afford different combinations of these crops and tillage methods.

The Spalding deep-tilling machine used in this experiment was received too late to prepare for wheat in 1911. The eight plots to be planted to corn were plowed on May 17, four of them deep and four shallow, as called for in the outline. The corn crop for this year follows the outline as regards depth of tillage, but was on land which was uniform with reference to crop sequence. The eight plots that should have been in wheat were fallow during the summer. They were plowed on July 13, four of them with the ordinary plow and four with the deep-tillage machine. Winter wheat was sown in the fall on four of the fallow plots and four of the plots that had been in corn. The preparation for the

crop of 1913 follows the outline in its entirety as regards depth of tillage. In the particular of crop sequence the four corn plots and the four wheat plots that should have followed wheat were on fallow land. Winter wheat was used in this experiment only the one year, durum wheat having been grown since the first crop.

Ordinary plowing has been done uniformly to the depth of 7 inches with a moldboard plow of the sulky type. Deep tilling has been done to the depth of 14 inches each year. Plowing for wheat has been done in the fall; plowing for corn has been done in the spring of each year, except in preparation for the crop of 1913.

The outline was departed from in preparing for the crop of 1916. The 4 corn plots that were to be sown to wheat were not plowed, but were double-disked in preparation for seeding. The 12 other plots—4 corn plots to be planted to corn, 4 wheat-stubble plots to be planted to corn, and 4 wheat-stubble plots to be planted to wheat—were all plowed 6 inches deep in the spring of 1916.

The results of this experiment for the six years 1911 to 1916, inclusive, are given in Table XVI. The yields given in this table are arranged under 16 heads: (1) Wheat following wheat on land deep-tilled each year, plot L; (2) wheat following wheat the first year after deep tillage on land deep-tilled every other year, plot J in the odd years and M in the even; (3) wheat following wheat the second year after deep tillage on land deep-tilled every other year, plot M in the odd years and J in the even; (4) wheat following wheat on land ordinary plowed each year, plot K; (5) wheat following corn on land deep-tilled each year, plot D in the odd years and N in the even; (6) wheat following corn the first year after deep tillage on land deep-tilled every other year, plot B in the odd years and O in the even; (7) wheat following corn the second year after deep tillage on land deep-tilled every other year, plot C in the odd years and P in the even; (8) wheat following corn on land ordinary plowed each year, plot A in the odd years and Q in the even. (9–16) Eight similar combinations of crop sequence and tillage method occur with the corn crop.

At the right of Table XVI are two averages. The first needs no explanation, being the average of each method for the entire period of years. Under the corn crop the grain average is the average of the three years when grain was produced and the fodder average is the average of the total weights for the three years when little or no grain was produced. The second average is the average of the two crop sequences on similar conditions of depth of cultivation.

The results given in Table XVI show a rather striking effect of crop sequence. Wheat following corn and corn following corn are both markedly better than the same crops following wheat. This positive result is the more striking when considered in connection with the lack of difference in the average yields resulting from the very marked dif-

ferences in the depth of plowing. Where wheat follows wheat the three combinations, (1) deep-tilled each year, (2) the first crop after deep tillage, and (3) the second crop after deep tillage on plots alternately deep- and shallow-tilled, exhibit no difference. The plot that received no deep tillage shows an apparent increase over any of these combinations, but a careful analysis of the results of the four plots from year to year points very strongly to the belief that this apparent increase may be within the limits of the experimental error.

TABLE XVI.—*Yields at Akron (Colo.) Field Station of wheat and corn in deep-tillage experiment for the years 1911 to 1916, inclusive*

Crop and treatment.	Previous crop.	Plot.[a]	Yield (bushels per acre).[b] 1911	1912	1913	1914	1915	1916	Averages. Grain.	Averages. Fodder.	Similar treatment after both wheat and corn.[c] Grain.	Similar treatment after both wheat and corn.[c] Fodder.
WHEAT.												
Deep tillage each year.	Wheat.	L	......	22.5	0.3	17.1	25.5	3.5	13.8	......	16.2	......
First crop after deep tillage.	...do....	J-M	......	22.9	.3	16.4	24.5	3.2	13.5	......	18.0	......
Second crop after deep tillage.	...do....	M-J	......	29.2	.2	14.8	22.7	2.1	13.8	......	18.7	......
Ordinary plowing...	...do....	K	......	32.8	.3	17.7	22.4	2.7	15.2	......	21.0	......
Deep tillage each year.	Corn...	D-N	......	33.0	3.5	18.7	29.6	8.1	18.6	......	......	......
First crop after deep tillage.	...do....	B-O	......	40.0	9.6	21.9	33.3	7.7	22.5	......	......	......
Second crop after deep tillage.	...do....	C-P	......	45.9	5.3	28.1	30.9	7.6	23.6	......	......	......
Ordinary plowing...	...do....	A-Q	......	54.7	10.5	31.9	30.0	6.4	26.7	......	......	......
CORN.[b]												
Deep tillage each year.	Corn...	F	1,000	45.2	2,320	20.1	35.6	1,360	33.6	1,560	29.3	1,606
First crop after deep tillage.	...do....	H-E	864	40.7	2,320	16.9	27.0	1,600	28.2	1,595	26.5	2,080
Second crop after deep tillage.	...do....	E-H	432	41.7	1,960	17.7	36.3	1,160	31.9	1,184	26.9	1,511
Ordinary plowing...	...do....	G	720	43.1	2,120	16.6	40.9	1,400	33.5	1,413	28.9	1,666
Deep tillage each year.	Wheat.	N-D	1,456	32.3	2,100	9.8	32.8	1,400	25.0	1,652	......	......
First crop after deep tillage.	...do....	P-C	3,456	37.1	2,760	10.9	26.3	1,480	24.8	2,565	......	......
Second crop after deep tillage.	...do....	O-B	1,904	21.7	2,090	11.2	32.7	1,520	21.9	1,838	......	......
Ordinary plowing...	...do....	Q-A	1,904	29.8	2,810	8.7	34.2	1,040	24.2	1,918	......	......

[a] Where two plots appear under the same heading, the crop is on the first one in the odd years and on the second in the even years.
[b] For three years, 1911, 1913, and 1916, when little or no grain was produced the yield of corn is in total pounds per acre.
[c] Example: Average yield of wheat for deep tillage after both wheat and corn is 13.8+18.6÷2=16.2.

In the four plots where wheat follows corn there is a more pronounced evidence of a positive result. The heaviest yield has been on the ordinary-plowed plot, the next heaviest from the second year after deep tillage, the third heaviest from the first year after deep tillage, and the

lowest yield from the plot deep-tilled every year. This relation has been quite consistent during three of the five years for which results have been obtained. During the two other years little difference as a result of the different preparations is exhibited.

From the corn crop no significant differences as a result of different depths of tillage have been obtained. Between the average of the two plots deep-tilled each year and the average of the two plots ordinary-plowed each year there is as an average of six years' results a difference of only 0.4 bushel of corn in favor of one and 60 pounds of fodder in favor of the other. In the three years when grain was produced both of these averages exceeded those of the plots alternately deep-tilled. In the three years in which fodder only was produced, the yields of these plots exceeded those of one of the alternately deep-tilled plots, but were in turn exceeded by that of the other.

That these differences are accidental rather than due to the effect of the tillage method is shown by the fact that they are determined, in at least a part of the cases, by differences in 1911 in the yield of plots exactly similar in their preparation. The only differences of cultivation or sequence that year were that the four plots F, E, D, and C, being the four which appear under the headings "Deep tillage each year" and "First crop after deep tillage," were deep-tilled, while the other four plots were given ordinary plowing. Two of the deep-tilled plots and two of those shallow-plowed produced some grain, while the other four did not. The yields are given, however, as total weights of fodder. In no other year has there been observed in plots of different treatment such great differences as were evidenced this year between plots of similar treatment.

From the evidence presented by this experiment it is safe to say that at this station deep tillage has no efficacy either in overcoming drouth or in increasing the yields of wheat or corn in the average of a series of years. There is, indeed, strong indication that the yields of wheat may be materially reduced by this practice. The conclusiveness of this evidence is strengthened by its general agreement with the results of the shorter experiment in the use of dynamite and the longer and more extensive experiment with subsoiling.

ARDMORE FIELD STATION

DEEP TILLAGE BY THE USE OF DYNAMITE

The deep tillage with dynamite experiment at Ardmore, S. Dak., is similar to the one at the Judith Basin Field Station, and in every particular except the size of the plots and their grouping in the field is the same as the deep-tillage experiment at Akron. Figure 4 illustrates the manner in which the plots are laid out. In preparation for the crop raised in the odd years the two center tiers of plots running north and south are dynamited. In preparation for the crop raised in the even

years the two center tiers of plots running east and west are dynamited. In the odd years the two north tiers of plots are cropped to wheat and the two south tiers to corn. In the even years the two east tiers of plots are cropped to wheat and the two west tiers are cropped to corn. The size of each plot is ¼ acre.

Eight plots were dynamited late in September, 1912. The charges of powder were placed 15 feet apart in each direction, ½ pound of 20 per cent powder being used in each charge. The charges were fired at a depth of 30 inches, which is as deep as is practicable to place them in this soil when it is dry. The soil on which this experiment is located is of the heaviest clay of the type commonly known as gumbo. Apparently it is almost impervious to water under some conditions, and samples taken to a depth of 10 feet reveal no change in its character. After the explosive had been used, an examination of the soil showed that it had not been in the least affected at a distance greater than 5 feet from the location of the charge. In many cases the soil was not disturbed beyond a distance of 3 feet from the charge. This was when the soil was very dry. When the soil is at all moist, the powder exerts a packing rather than a disrupting effect.

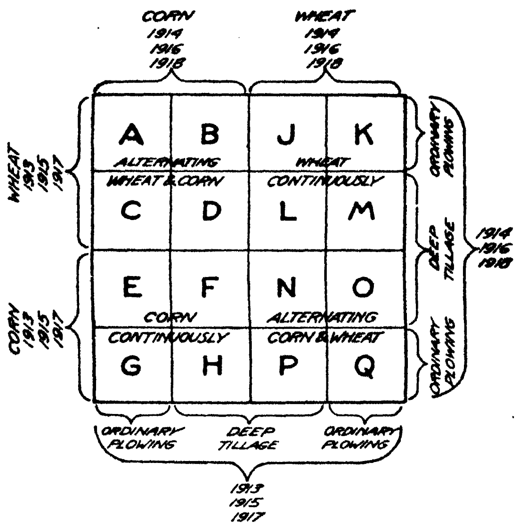

FIG. 4.—Diagram of plots in dynamiting experiment at Ardmore, S. Dak.

After the first year the powder company withdrew its active cooperation, but the experiment has been continued by the Department, and the blasting done each year as provided for in the original outline.

Each fall, after the dynamiting is completed, the entire 16 plots are plowed to a depth of 7 or 8 inches. They are left rough to overwinter, the object being to leave them in condition to catch and retain the maximum amount of snow and rainfall. In the spring the plots are given the necessary tillage with a disk and a harrow to put them in proper conditions for seeding.

In 1913 good stands of both corn and wheat were obtained. This land had produced a crop of sorghum in 1912, and consequently contained no water in storage. The season of 1913 was very dry, particularly during

June, July, and August. The wheat reached a height of only about 10 inches and probably would not have yielded as much as 1 bushel per acre. It was clipped with a mower and raked off the ground, no attempt being made to obtain yields. No appreciable difference could be observed between the plots as a result of the dynamiting. The corn crop suffered badly from drouth and did not ear. It was severely damaged by hail, and the yields were not determined. No appreciable difference could be observed between any of the plots.

In 1914 a very promising crop of wheat was destroyed on June 24 by a heavy hailstorm. The corn suffered from this storm, and again from a second one on July 7. Hail in August again damaged it to a limited extent. During the latter part of the season it suffered from lack of water and was cut for fodder on August 18. No ears were produced. Both 1915 and 1916 were productive of good crops of both wheat and corn.

Table XVII gives the yields from this experiment. They are arranged and averaged the same as the data in Table XVI, which reports the results of the deep-tillage experiment at Akron.

TABLE XVII.—*Yields at Ardmore (S. Dak.) Field Station of wheat and corn in dynamiting experiment for the years 1914 to 1916, inclusive*

Crop and treatment.	Previous crop.	Plot.[a]	Yield (bushels per acre).				
			1914 [b]	1915	1916	Average.	Average of similar treatment after both wheat and corn.
WHEAT.							
Dynamite each year	Wheat	L	......	26.9	15.9	21.4	21.0
First crop after dynamite	...do	J-M	......	35.3	11.7	23.5	22.2
Second crop after dynamite	...do	M-J	......	35.7	18.7	27.2	28.3
Ordinary plowing	...do	K	......	31.5	16.1	23.8	23.0
Dynamite each year	Corn	D-N	......	25.7	15.3	20.5	......
First crop after dynamite	...do	B-O	......	26.8	14.7	20.8	......
Second crop after dynamite	...do	C-P	......	39.8	19.0	29.4	......
Ordinary plowing	...do	A-Q	......	26.4	18.0	22.2	......
CORN.							
Dynamite each year	Corn	F	632	17.4	14.7	16.1	18.7
First crop after dynamite	...do	H-E	716	40.4	15.6	28.0	26.2
Second crop after dynamite	...do	E-H	634	29.4	14.3	21.9	22.6
Ordinary plowing	...do	G	824	33.9	22.3	28.1	29.2
Dynamite each year	Wheat	N-D	860	24.1	18.2	21.2	......
First crop after dynamite	...do	P-C	672	35.3	13.3	24.3	......
Second crop after dynamite	...do	O-B	1,168	28.1	18.2	23.2	......
Ordinary plowing	...do	Q-A	1,180	40.6	20.0	30.3	......

[a] Where two plots appear under the same heading, the crop is on the first one in the odd years and on the second in the even years.
[b] Weight of fodder; no grain produced.

With the wheat crop the difference, if any, between dynamiting each year, the first year after dynamiting, and not dynamiting at all appears to be slightly in favor of the latter. The highest yields have been obtained each year both where wheat follows wheat and where wheat follows corn on plots the second year after dynamiting. The fact that these results have been obtained from four separate plots would seem to remove it from the possibility of being due to soil variation. With the corn crop the tendency has been for the land not dynamited at all to produce the highest yields. The second highest average yield has been the first year after dynamiting, the third highest the second year after dynamiting, while the lowest yields have been from those plots dynamited each year.

The results of both crops together indicate that it is very questionable whether any actual increase of yields may be obtained on this soil as a result of dynamiting. There can be no question, however, of the conclusion that there is no chance of yields being increased sufficiently to make the operation a profitable one. The experiment is being continued, however, and has been somewhat extended. In order to determine the effect of a complete loosening of the soil regardless of cost, one one-tenth acre plot was dynamited in the fall of 1915 with charges set close enough together to insure the loosening and stirring of all the soil on the plot, the charges being fired at a depth of 30 inches. In 1916 the appearance and the yield of the wheat on this plot was practically the same as that on an adjoining plot that was ordinary fall-plowed.

BELLEFOURCHE FIELD STATION

DEEP TILLAGE BY THE USE OF DYNAMITE

In October, 1912, a representative of the powder company gave a demonstration in blasting soil for field crops at the Bellefourche Field Station. A one-tenth acre plot (plot 1 in Series VIII, field B), 8 rods by 2 rods, that had been in millet (*Panicum miliaceum*) was selected for the demonstration. The shots were placed 20 feet apart each way and 3 feet deep. This plot and two adjoining plots that were used for controls had been plowed shortly before the dynamiting. The control plots (plots 1 in Series VII and Series IX) adjoined the ends of the dynamited plots, one on the east and one on the west. The soil on the plots in Series VII and VIII is uniform, but that on the plot in Series IX is poorer on account of a hardpan spot covering nearly half its area. This plot was manured.

In the spring of 1913 all three plots were treated alike and seeded to Sixty-Day oats. The dynamiting and manuring were not repeated but the plots were given uniform treatment and again seeded to oats in 1914. Both seasons spring conditions were favorable, but after the latter part of June or the first part of July the crop suffered from drouth.

In 1915 all the plots were given the same treatment and seeded to wheat, for which the season proved extraordinarily favorable. The plots were all fallowed in 1916 and seeded to alfalfa in 1917. The yields for the three years following the dynamiting are shown in Table XVIII.

TABLE XVIII.—*Yields at Bellefourche (S. Dak.) Field Station of oats and wheat in dynamiting experiment for the years 1913, 1914, and 1915*

Plot No.	Treatment.	Yield in bushels each year.		
		1913, oats.	1914, oats.	1915, wheat.
B VII–1	Fall-plowed	25.9	19.1	58.8
B VIII–1	Fall-plowed and dynamited	18.4	18.8	54.0
B IX–1	Fall-plowed and manured	24.1	16.1	52.8

The only conclusion that can be drawn from these yields is that dynamiting is not effective in increasing yields. The first year after dynamiting the effect appears to have been quite the opposite. It is impossible to say how much effect the manure used on Plot IX–1 had in overcoming the initially poor condition of that plot.

JUDITH BASIN FIELD STATION

DEEP TILLAGE BY THE USE OF DYNAMITE

An experiment similar to those at Akron and Ardmore, in the use of dynamite as a medium of deep tillage was inaugurated in the fall of 1912 at the Judith Basin Field Station. The plots are ¼ acre in size. The plan of the experiment is identical with that at Ardmore, as shown in figure 4 and described in connection with the results from that station.

The land on which this experiment was started was prairie sod broken in June, 1909, and seeded to winter wheat late in November of that year. A poor crop of wheat was harvested from the land in 1910, the yield being 10 bushels per acre. The land was plowed in the spring of 1911 and seeded to flax, which yielded 12 bushels per acre. The Judith Basin Field Station obtained possession of this tract of land in November, 1911. The field was bare -fallowed during 1912, being plowed late in June, and double-disked and harrowed three times during the remainder of the season. The plots were staked out, and the first dynamiting was done in September of that year. The entire block of 16 plots was plowed in the spring of 1913 and seeded to wheat and corn, as called for in the outline of the experiment. Very little difference could be noted in the growth of grain on the different plots during the season. At harvest time the plots were as uniform in growth and height as if they had all received the same treatment.

In preparation for the crop of 1914 dynamiting was done in the fall, but all plowing was deferred until spring. Good stands were obtained with both wheat and corn and good crops produced.

In preparation for the crop of 1915 the dynamiting was again done in the fall and the plowing in the spring. In preparing for the crop of 1916 both dynamiting and plowing were done in the fall. The plowing remained rough over the winter. In the spring all plots were double-disked and harrowed in preparation for seeding. As in previous years, no variation in growth that could be attributed to the use of dynamite could be observed in the plots in the field.

The yields from the 16 plots in this experiment for the four years 1913 to 1916, inclusive, are presented in Table XIX. The arrangement of the data in this table is the same as in Tables XVI and XVII. The yields of corn are given in total pounds of fodder per acre. The corn used in these experiments is raised for fodder at this station and no grain produced.

TABLE XIX.—*Yields at Judith Basin Field Station of wheat and corn in dynamiting experiment for the years 1913 to 1916, inclusive*

Crop and treatment.	Previous crop.	Plot.[a]	Yield.					Average of similar treatment after both wheat and corn.
			1913	1914	1915	1916	Average.	
WHEAT.								
Dynamite each year...	Wheat.	L	31.1	20.2	30.6	18.8	25.2	26.2
First crop after dynamite.	...do...	J-M	33.2	18.6	32.1	19.7	25.9	26.4
Second crop after dynamite.	...do...	M-J	30.8	17.3	29.8	19.4	24.3	25.9
Ordinary plowing.....	...do...	K	30.6	17.0	30.1	17.1	23.7	23.4
Dynamite each year...	Corn...	D-N	31.2	20.6	32.6	24.0	27.1	......
First crop after dynamite.	...do...	B-O	31.2	20.1	32.1	23.7	26.8	......
Second crop after dynamite.	...do...	C-P	27.9	23.2	32.3	26.4	27.5	......
Ordinary plowing.....	...do...	A-Q	26.7	17.3	27.9	20.6	23.1	......
CORN.[b]								
Dynamite each year...	Corn...	F	2,640	9,700	2,115	4,400	4,714	3,903
First crop after dynamite.	...do...	H-E	2,240	8,500	2,120	3,400	4,065	3,309
Second crop after dynamite.	...do...	E-H	2,880	8,700	1,695	4,600	4,469	3,998
Ordinary plowing.....	...do...	G	2,200	8,400	1,840	4,400	4,210	3,363
Dynamite each year...	Wheat.	N-D	2,580	5,700	1,685	2,400	3,091	......
First crop after dynamite.	...do...	P-C	2,480	3,400	1,530	2,800	2,553	......
Second crop after dynamite.	...do...	O-B	2,440	6,900	2,165	2,600	3,526	......
Ordinary plowing.....	...do...	Q-A	1,880	3,900	1,480	2,800	2,515	......

[a] Where two plots appear under the same heading, the crop is on the first one in the odd years and on the second in the even years.

[b] Yields of corn in pounds of fodder. No grain produced.

In the results from the wheat crop no difference is exhibited between the yields from the plots dynamited each year and from those plots dynamited every other year either where wheat follows wheat or where wheat follows corn. All of these apparently have an advantage of about 3 bushels per acre over the plots not dynamited at all.

The yields from the corn crop exhibit a marked effect as a result of crop sequence, the average yield following corn being nearly ¾ ton per acre greater than the average yield following wheat. Every plot following corn has outyielded every plot following wheat. No such marked effect or consistency of results is to be noted as a result of dynamiting. The yield of the plot dynamited each year has been practically the same as the yield the second year after dynamiting on the plot alternately dynamited and not dynamited. Both of these yields have been about 600 pounds per acre greater than those from the plot not dynamited at all and from the first year after dynamiting on the plot alternately dynamited and not dynamited. This inconsistent combination of results indicates very strongly that the variations are accidental rather than due to the effects of dynamiting.

Granting the proposition, which is by no means conclusively proved by the data at hand, that yields may be slightly increased by the use of dynamite, the possible increase is too small to hold out any hope of the operation being profitable even if the effect of the dynamite persisted for a considerable number of years.

SUMMARY OF RESULTS OF DEEP TILLAGE BY THE USE OF DYNAMITE OR SPECIAL PLOWS

In summation of the results from all stations it seems very questionable that deep tillage either by the use of special plows or dynamite has been effective in increasing yields. The most favorable evidence is with corn the second year after dynamiting at Akron; with wheat the second year after dynamiting at Ardmore; and with wheat after dynamiting at Judith Basin. The apparent increases in these cases are small and are offset by losses so that the averages of all trials with both crops show no increases over ordinary plowing.

Deep tilling by these methods, as well as by subsoiling, has been of no value in overcoming drouth.

The results offer no hope of profitably increasing the yield of either wheat or corn by means of deep tillage.[1]

RESULTS OF OTHER INVESTIGATIONS OF SUBSOILING AND DEEP TILLING

UTAH.—Experimental work has been conducted cooperatively at the Nephi, Utah, Substation since 1907 by the Office of Cereal Investigations of the Bureau of Plant Industry and the Utah Agricultural Experiment Station.

[1] These conclusions are supported and strengthened by the results of 1917, which was a year of low yields owing to drouth.

Cardon (*1*) summarizes the results of five years' work with deep tillage for winter wheat as follows:

The results of five years show that there was no advantage in deep plowing or subsoiling over shallow plowing so far as moisture conservation is concerned. There was no material difference in the yields obtained from plats plowed at different depths, varying from 5 to 18 inches. The highest average yield was obtained from plats plowed 10 inches deep, and the lowest average yield was from the plats subsoiled 18 inches deep, while the 5-inch plowing yielded higher than the 15-inch subsoiling.

ILLINOIS.—Mosier and Gustafson (*4*) report the results of investigations in Illinois as follows:

Investigations to determine the value of subsoiling in preparation for corn on gray silt loam on tight clay, the common prairie soil of the lower Illinois glaciation, have been carried on for eight years at the Odin Field, in southern Illinois. * * * With every soil treatment there was an almost uniform decrease in yield for subsoiling. The general average for eight years shows a decrease of 2.7 bushels per acre. The alleged benefit of subsoiling is the increasing of the water capacity of soils and of their ability to retain water during dry seasons. Yet in 1913 and 1914, both of which were very dry seasons, this method, as a general average, gave only the very slight increase of .5 and .7 bushels respectively. The subsoil was loosened by the plow, but ran together as soon as it was wet and became approximately as it was before. The experiments as a whole show that subsoiling on this type of soil not only does not pay, but is a losing operation, for in order to pay for the extra work involved in subsoiling, at least a three-bushel increase would be necessary.

Under the head of "Deep tilling" in the same bulletin the authors present no data of yield, but make the following statement:

Farmers are frequently urged to purchase a machine for plowing to a depth of 12 to 15 inches. There is little doubt that under certain conditions of soil and climate such plowing would be beneficial; but the results obtained by the Experiment Station in this state with the deep-tilling machine on the common prairie soil of the corn belt do not warrant recommending its purchase.

PENNSYLVANIA.—Noll (*5*) summarizes the result of three years' trial of the Spalding deep-tillage machine on the farm of the Pennsylvania State College as follows:

The soil in which this experiment was conducted is of the Hagerstown series. It varies in texture from clay loam to gravelly silt loam, but is chiefly clay loam. The surface soil is so deep that in most of the area little of the clayey subsoil was turned up. The soil is well drained.

Eight plats 35.5 ft. wide, varying in length from 902.5 ft. to 1,000 ft. were plowed at first. These were later made 957.2 ft. long and comprised .78 of an acre each.

Timothy sod was plowed for corn in the fall of 1909 and the spring of 1910, two plats being plowed with each implement in the fall and two in the spring.

In the fall of 1910 and the spring of 1911 the corn stubble land was plowed in the same way, and in the spring four plats were seeded to oats and four to beardless barley and alfalfa.

In the fall of 1911 the four plats which had received oats were plowed and seeded to wheat, two plats being plowed with each implement.

The crops for which the plowing was done were corn, oats, barley, wheat, and alfalfa, each one year.

Under the conditions named above the two kinds of plowing gave practically the same results for all the crops grown.

MISSISSIPPI.—Ricks (*6*), reporting the results of subsoiling with plow and with dynamite at the Central Mississippi Station, shows the following corn yields:

In 1913, not subsoiled, 31.8 bushels; subsoiled with plow, 25.5 bushels; subsoiled with dynamite, 27.7 bushels. In 1914 the yields were: Not subsoiled, 30 bushels; subsoiled with plow, 27.2 bushels; subsoiled with dynamite, 29.1 bushels. He says:

These plats were on a Houston clay soil of medium fertility. The subsoiling was done in March of 1913. The check plats were broken about seven inches deep. . . . . Subsoiling for corn, as well as for any other crop gives us no returns.

The same author (*7*) in describing the preparation of the soil for alfalfa says:

Good deep plowing where there is good drainage has given us as satisfactory results as subsoiling with dynamite or with a subsoil plow.

This is under an annual rainfall of about 60 inches.

TEXAS.—Hastings and Letteer (*2*) in reporting on the experiments in subsoiling at San Antonio, Tex., covering three years, 1910, 1911, and 1912, conclude that—

(1) Subsoiling is an expensive practice and so adds to the cost of preparation for a crop that unless materially increased yields result it can not be profitably adopted as a regular farm practice.

(2) Subsoiling has been tested at the San Antonio Experiment Farm for three years in rotation experiments with corn, cotton, and oats for hay and for grain.

(3) The yields of corn, cotton, and oats for hay and for grain have been either slightly increased or slightly decreased on subsoiled land. In no instance has the difference been significant.

(4) The depressing residual effect of subsoiling on the yields of corn and cotton was most marked when the crop was planted from 1 to 8 months after subsoiling; 15 months after subsoiling but little depressing effect was noted.

(5) In the soil-moisture studies so far made at San Antonio it has been found that subsoiling has not increased the moisture content of the soil.

(6) The results of these tests indicate that since neither the moisture content of the soil nor the yields of corn, cotton, and oats are increased by subsoiling, the practice is not advisable in connection with the crops mentioned in the San Antonio region of Texas.

RUSSIA.—Rotmistrov (*8*), in discussing the state of the drouth question, says:

Deep mellowing of the soil which all the writers on this subject unanimously regard as a matter of great importance with regard to fighting against drouth, has also very little real significance. On the Odessa field there have been more than 1,000 experiments made on the effect of deep plowing for winter and spring crops, and no difference in favor of deep [10½ inches] or even mediate [7 inches] plowing was obtained in the harvest. Investigations into the humidity of the soil also showed no difference in that respect between deep and shallow [3½ inches] plowing.

The argument in favor of deep plowing, that deeply mellowed soil imbibes more atmospheric residue [precipitation], falls through, because little residue settles on the steppes districts and it all enters the soil whether deeply plowed or not. On certain types of soil and in more northern regions deep plowing may have a beneficial effect for other reasons—airing the soil, etc.—but not as regards opposing drouth. [TRANSLATION.]

SUMMARY

Subsoiling, deep tilling, and soil dynamiting are all operations that increase the expense of production over that on ordinary plowing. They also increase the amount of labor expended on a given area, or reduce the acreage that can be prepared by a given working unit. Subsoiling is as laborious and expensive an operation as plowing, but must be done in addition to it and at the same time. Plowing with a special deep-tillage machine to a depth of 12 to 14 inches requires considerably more than double the labor, time, and expense of ordinary plowing. The use of dynamite in the least quantity that might be effective involves an added expense for material and labor of more than $20 per acre. Consequently, in order to justify their use, these practices should show increases in yields sufficient to pay for the extra expense involved.

In any year a combination of conditions favorable to subsoiling may occur at any station. At some stations the average results of a series of years shows no measurable effect on crop yields as a result of subsoiling. At other stations the effect has clearly been to decrease yields. At still other stations, particularly at Hays, Kans., subsoiling appears to have resulted in significant increases in crop yields. With some of the crops showing increases, however, the yields from either method have been too small to be profitable.

Recognizing the fact that there may be times and places giving results favorable to subsoiling or other methods of deep tilling, the average yields obtained in the extensive experiments here reported seem to warrant the conclusion that as a general practice for the Great Plains as a whole no increase of yields or amelioration of conditions can be expected from the practice.

In their relative response to deep tillage there is no marked difference to be observed between crops.

Subsoiling and deep tilling have been of no value in overcoming drouth. The effect, on the contrary, apparently has been to reduce the yields in those seasons that are below the average in production.

Experiments have been conducted with the subsoil plow, the Spalding deep-tillage machine, and dynamite. The effect or lack of effect of deep tillage appears to be essentially the same, irrespective of the means by which it is accomplished.

These conclusions are the result of extensive experiments covering a wide range of crops, soils, and conditions in the Great Plains. Experiments conducted in the Great Basin under semiarid conditions with the greater part of the precipitation occurring in the winter; under humid conditions in the States of Illinois, Pennsylvania, amd Mississippi; under semiarid conditions at San Antonio, Tex.; and under semiarid conditions on the black soil of southern Russia have all led to the same con-

clusion: that yields can not be increased nor the effects of drouth mitigated by tillage below the depth of ordinary plowing.

The quite general popular belief in the efficiency of deep tillage as a means of overcoming drouth or of increasing yields has little foundation of fact, but is based on misconceptions and lack of knowledge of the form and extent of the root systems of plants and of the behavior and movement of water in the soil.

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OVERWINTERING OF THE CITRUS-CANKER ORGANISM IN THE BARK TISSUE OF HARDY CITRUS HYBRIDS[1]

COOPERATIVE INVESTIGATIONS BETWEEN THE DEPARTMENT OF PLANT PATHOLOGY, ALABAMA AGRICULTURAL EXPERIMENT STATION, AND THE OFFICE OF CROP PHYSIOLOGY AND BREEDING INVESTIGATIONS, BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE

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During the course of our field inoculation experiments conducted in southern Alabama in the fall of 1917 to determine the resistance and susceptibility to Citrus-canker of some of the wild relatives, Citrus fruits, and the more common hybrids of the genus Citrus, a most interesting feature has recently developed in connection with the ability of the organism to survive the winter in the outer bark tissue of some of these plants.

All the plants were set in the isolation field in July, 1917, extreme precautions surrounding the experiments being maintained. By the middle of September they had made a rapid growth and were at that time in fine shape for inoculation.

Included in a series of inoculations made on September 16, 1917, were the hybrids Rusk citrange (CPB 7956A),[2] Savage citrange (CPB 7961), and citrandarin (CPB 40175A), and two plants each of *Poncirus trifoliata*, grapefruit (*Citrus grandis*), and Satsuma orange (*Citrus nobilis* var. *unshiu*. In making the inoculations 100 cc. of a 48-hour culture of *Pseudomonas citri* in beef bouillon were thoroughly sprayed on each plant by means of an atomizer.

Although repeated observations were made during October and November of the plants enumerated above, only *P. trifoliata* and grapefruit showed any evidence of canker infection, and this only occurred to a slight extent on the foliage. It was thought that absence of infection on the Rusk and Savage citranges, as well as on the citrandarin and other plants, could be in part accounted for by the unfavorable temperature prevailing at the time the inoculations were made. This view was also somewhat strengthened by the fact that the more susceptible plants, such as grapefruit and *P. trifoliata* revealed only a minimum amount of infection two months after making the inoculations. With such unfavorable temperatures prevailing because of the lateness of the season no positive results were obtained with the hybrids, particularly with the citranges and citrandarins.

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[1] Published with the approval of the Director of the Alabama Agricultural Experiment Station.
[2] CPB=Crop Physiology and Breeding Investigations.

Notwithstanding the fact that all the plants were carefully observed at intervals throughout the winter, no infection was found on the hybrids, although these were in a thrifty condition, with an abundance of healthy foliage.

On April 2, 1918, positive evidence of Citrus-canker infection was observed on the Rusk and Savage citranges, as well as on the citrandarin. The plants of *P. trifoliata* (Pl. 58, B) that were inoculated at the same time (September 16, 1917) also revealed new infections. Unfortunately the grapefruit and Satsuma plants were killed by the low temperatures prevailing during the winter months, and no further data could be obtained here. The hybrid plants were heavily infected, the infection in each case being confined to the main stem and branches (Pl. 58, A, C, D). The infection appeared simultaneously and extensively on all the twigs, branches, and main stems of the plants. Although the foliage was very healthy and apparently active and had been so throughout the winter, no sign of infection was observed on the leaves.

Cankered twigs from the Rusk and Savage citranges, the citrandarin, and *C. trifoliata* were collected and taken to the laboratory to ascertain whether the organism was viable and could be recovered in culture. Within four days good colonies of the organism appeared on the plates, which left no question of their viability.

From the data at hand it would appear that the Citrus-canker organism is able to withstand the winter within the outer-bark tissues of the host. Wolf [1] states that the lenticels probably serve as portals of entrance for the organism into the stems, and from the results it would appear that this view is entirely possible. The organism probably gains entrance into the outer-bark tissue through the lenticels and remains dormant through the winter months. On the return of more favorable conditions of temperature, humidity, and rapid growth of the plant, the canker organism becomes active.

The weather records in this vicinity during the fall and winter of 1917–18 reveal a minimum temperature of 15.5° F. It would seem, therefore, that the bacteria which gained entrance into the outer-bark tissues, probably through the lenticels, at the time of inoculations, September 16, 1917, were offered sufficient protection to withstand the above temperature, whereas the foliage infections were completely killed or their virulency lowered to such an extent that infection was not possible.

From the fact that the Citrus-canker organism is able to withstand such a low temperature and remain in a dormant condition for 6½ months in the outer-bark tissues of the twigs and branches, extreme care and caution must be exercised in the use of Citrus plants from canker-infected regions in the selection of budwood from nurseries and orchards in which canker has been found within a year, in the length of the quarantine period, and in the complete eradication of Citrus-canker from nurseries and orchards, especially in plantings of *P. trifoliata*.

[1] WOLF, F. A. CITRUS-CANKER. *In* Jour. Agr., Research, v. 6, no. 2, p. 79. 1916.


A
B
C
D

PLATE 58

Citrus-canker spots on twigs from plants in the isolation field, inoculated on September 16, 1917. These first appeared on April 2, 1918. Photographed on May 2, 1918.

A.—Citrandarin (CPB 40175A).

B.—*Poncirus trifoliata* (seedling, Alabama).

C.—Savage citrange (CPB 7961).

D.—Rusk citrange ʻCPB 7956A).

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RESISTANCE OF SEEDS TO DESICCATION

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INTRODUCTION

The question of the resistance of seeds to extreme drying probably first came into prominence as a result of the use of artificial desiccation to hasten afterripening, as reported by Hotter, Nobbe, Atterberg, Kiessling, and others. The drying was usually done in drying ovens, and the temperatures used frequently were not such as to reduce the moisture content of the seeds below 5 or 6 per cent. Sometimes, however, when higher temperatures were used, the moisture content fell considerably lower than this, and in such cases the germination was often lowered. The question, of course, arises whether the injury comes from the loss of moisture or as the effect of the high temperatures used.

HISTORICAL REVIEW

Schröeder [1] dried barley and wheat for 12 weeks over sulphuric acid. At the end of this time they contained only 1 to 2 per cent of moisture, but nearly all germinated. Nobbe [2] reduced the water content of rye to 1.2 per cent by drying it at 80° C. with very little effect upon subsequent germination. More severe drying by heat seriously injured the germination of rye, and less severe drying had an injurious effect upon wheat and oats. About the same time Ewart,[3] working with seeds of wheat, corn, barley, peas, haricots, hemp, squash, rape, and sunflower, which he dried in a vacuum desiccator at 37 to 38° C., concluded that it is impossible to reduce the percentage of water held by even the most resistant seeds to lower than 2 or 3 per cent of their dry weight without injuriously affecting their vitality. Ewart's hypothesis was that excessive drying so changed the dormant protoplasm that upon being remoistened it was unable to reestablish the molecular groupings essential for normal vital activity.

[1] SCHRÖDER, G. ÜBER DIE AUSTROCKNUNGSFÄHIGKEIT DER PFLANZEN. *In* Untersuch. Bot. Inst. Tübingen, Bd. 2, Heft 1, p. 1–52. 1886.

[2] NOBBE, F. ÜBER KÜNSTLICHE GETREIDETROCKNUNG MIT BEZUG AUF DIE KEIMFÄHIGKEIT. *In* Mitt. Deut. Landw. Gesell., Jahrg. 12, Stück 14, p. 185–186. 1897.

[3] EWART, A. J. ADDITIONAL OBSERVATIONS ON THE VITALITY AND GERMINATION OF SEEDS. *In* Proc. and Trans. Liverpool Biol. Soc., v. 10, 1895/96, p. 185–193, 5 pl. 1896.

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Since Ewart published his papers, other investigators have put out results which seem to controvert his conclusions. Pickholz,[1] by storing over sulphuric-acid solutions and by drying over concentrated sulphuric acid *in vacuo*, varied the moisture content of Kentucky bluegrass seed from a mere trace to 32 per cent. The lower the moisture content at the time of beginning the germination test, the more rapid and complete was the germination at 20° C., though germination was little affected at 28°, or with a daily alternation between 20 and 28°. Seeds with a mere trace of water present germinated almost as well at the usually very unfavorable temperature 20°, as with the favorable alternation between 20° and 28°. Waggoner,[2] by drying first at 60° and later at 100° C., reduced the moisture content of radish seeds to 0.4 per cent without affecting subsequent germination.

EXPERIMENTAL WORK

The work of the present authors with seeds of a number of species of Gramineae corroborates the results of Pickholz and Waggoner. It should be noted, however, that some kinds of seeds are known to be unable to withstand even ordinary air-drying. Among these are the seeds of silver maple (*Acer saccharinum*), wild rice (*Zizania palustris*), the various species of willows (*Salix* spp.), and many water plants.

In the winter of 1913 seeds of Kentucky bluegrass (*Poa pratensis* L.) of 90 per cent germinating capacity were dried *in vacuo* over calcium oxid (CaO) at room temperature. With 1.5 per cent of moisture remaining in the seeds there was no fall in germinating capacity or in germinating energy. With 0.2 per cent moisture the germinating capacity remained the same, but the germinating energy was considerably less. With 0.1 per cent moisture the germinating capacity had fallen about 5 per cent, and the germinating energy was seriously reduced. One lot previously dried in a lime desiccator to 0.1 per cent of moisture was further dried in a vacuum oven at 100° C. for six hours to remove the last trace of moisture. The germinating energy (see below) was thus reduced to one-half what it was with 0.1 per cent of moisture, and the seedlings produced were weak; but the percentage of seeds which germinated remained the same as before removing the last 0.1 per cent of the water. All of the germination tests were conducted in the Jacobson apparatus with a daily alternation of temperatures between 20° and 30° C. All of the tests with a quick-germinating lot were continued for 28 days, and the ratio between the percentage of germination at the end

[1] PICKHOLZ, L. EIN BEITRAG ZUR FRAGE ÜBER DIE WIRKUNG DES LICHTES UND DER INTERMITTIERENDEN TEMPERATURE AUF DIE KEIMUNG VON SAMEN, SOWIE ÜBER DIE ROLLE DES WASSERGEHALTES DER SAMEN BEI DIESER WIRKUNG. *In* Ztschr. Landw. Versuchsw. Oesterr., Bd. 14, Heft 2, p. 124-151, 1 fig. 1911. Literaturverzeichnis, p. 150-151.

[2] WAGGONER, H. D. THE VIABILITY OF RADISH SEEDS (RAPHANUS SATIVUS L.) AS AFFECTED BY HIGH TEMPERATURES AND WATER CONTENT. *In* Amer. Jour. Bot., v. 4, no. 5, p. 299-313, 1 fig. 1917. Literature, p. 312-313.

of 7 days and that at the end of 28 days was used as a measure of the germinating energy. With a lot of seed which germinated more slowly all germination tests were continued for 35 days, and the ratio 14 to 35 days was used to express the germinating energy.

The series of experiments described in the following pages was begun on January 12, 1917. Two varieties of barley, *Hordeum vulgare* L.; two varieties of wheat, *Triticum aestivum* L. (*T. vulgare* Vill.); a sample of Sudan grass, *Holcus halepensis sudanensis* (Piper) Hitchcock (*Andropogon halepensis sudanensis* Piper); and one of Johnson grass, *Holcus halepensis* L. (*Sorghum halepense* Pers.) were stored at room temperature in evacuated desiccators over calcium oxid and over concentrated sulphuric acid. From the seeds in these desiccators and from control lots stored in an open vessel in a desk drawer samples were withdrawn for moisture determinations and germination tests at intervals during the next 10½ months. Germination tests of Johnson grass seed were made with a daily alternation of temperatures between 25° and 40° C. All tests were continued for from 9 to 17 days. Germination tests of all other seeds were made at 20° C., and the length of the tests varied from four to six days, germination being practically completed in four days. In all germination tests moist blotting-paper disks inclosed in 100-mm. petri dishes were used for germinating beds. The results of moisture determinations and germination tests are summarized in Tables I, II, and III.

TABLE I.—*Moisture content and percentage of germination of Svanhals barley and Johnson grass samples after storage for different lengths of time over lime and over sulphuric acid, compared with samples stored in an open vessel*

Date of test.	Length of time stored under different conditions.	Svanhals barley.					
		Moisture content (percentage of dry weight).			Germination (per cent).		
		Stored over calcium oxid.	Stored over sulphuric acid (sp. gr. 1.84).	Stored in open vessel.	Stored over calcium oxid.	Stored over sulphuric acid (sp. gr. 1.84).	Stored in open vessel.
1917.							
Jan. 12	....	....	....	9.1	....	....	99
Feb. 26	6 weeks...........	3.4	4.1	....	97	96	95
Apr. 2	11 weeks..........	2.7	2.9	....	96	99	....
May 3	16 weeks..........	1.5	1.8	8.2	97	100	99
June 19	23 weeks..........	1.7	1.9	11.8	95	94	98
July 12	6 months..........	1.1	1.3	12.1	96	98	98
Aug. 8	7 months..........	.7	.9	....	95	98	98
Sept. 5	8 months..........	....	....	....	97	97	97
Nov. 25	10½ months........	.8	.7	9.4	98	96	96

TABLE I.—*Moisture content and percentage of germination of Svanhals barley and Johnson grass samples after storage for different lengths of time over lime and over sulphuric acid, compared with samples stored in an open vessel*—Continued

Date of test.	Length of time stored under different conditions.	Johnson grass. Moisture content (percentage of dry weight). Stored over calcium oxid.	Stored over sulphuric acid (sp. gr. 1.84).	Stored in open vessel.	Germination (per cent). Stored over calcium oxid.	Stored over sulphuric acid (sp. gr. 1.84).	Stored in open vessel.
1917.							
Feb. 26	6 weeks	3.3	3.2	....	....	....	....
Apr. 2	11 weeks	1.8	2.1	....	58	42	65
May 3	16 weeks	1.3	1.6	8.4	64	64	66
June 19	23 weeks	1.2	1.6	11.7	47	58	58
July 12	6 months	.6	.8	11.5	65	56	56
Aug. 8	7 months	.4	.5	....	48	48	68
Sept. 5	8 months	....	....	....	52	60	78
Nov. 25	10½ months	.1	.2	9.3	80	78	82

TABLE II.—*Moisture content and percentages of germination of White Smyrna barley and Sudan grass samples after storage for different lengths of time over lime and over sulphuric acid, compared with samples stored in an open vessel*

Date of test.	Length of time stored under different conditions.	White Smyrna barley. Moisture content (percentage of dry weight). Stored over calcium oxid.	Stored over sulphuric acid (sp. gr. 1.84).	Stored in open vessel.	Germination (per cent). Stored over calcium oxid.	Stored over sulphuric acid (sp. gr. 1.84).	Stored in open vessel.
1917.	*Months.*						
Jan. 12	....	....	....	9.3	....	....	87
July 12	6	1.1	1.3	12.2	88	88	92
Aug. 8	7	.7	.7	....	87	90	91
Sept. 5	8	....	....	....	84	88	86
Nov. 25	10½	.6	.6	9.5	85	86	82

Date of test.	Length of time stored under different conditions.	Sudan grass. Moisture content (percentage of dry weight). Stored over calcium oxid.	Stored over sulphuric acid (sp. gr. 1.84).	Stored in open vessel.	Germination (per cent). Stored over calcium oxid.	Stored over sulphuric acid (sp. gr. 1.84).	Stored in open vessel.
1917.	*Months.*						
Jan. 12	....	....	....	....	....	....	90
July 12	6	0.9	1.2	12.1	92	94	92
Aug. 8	7	.6	....	....	81	94	94
Sept. 5	8	....	....	....	86	91	92
Nov. 25	10½	.6	.5	9.7	90	92	95

TABLE III.—*Moisture content and percentages of germination of samples of wheat after storage for different lengths of time over lime and over sulphuric acid, compared with samples stored in an open vessel*

Date of test.	Length of time stored under different conditions.	Kharkof wheat. Moisture content (percentage of dry weight). Stored over calcium oxid.	Stored over sulphuric acid (sp. gr. 1.84).	Stored in open vessel.	Germination (per cent). Stored over calcium oxid.	Stored over sulphuric acid (sp. gr. 1.84).	Stored in open vessel.
1917.	*Months.*						
Jan. 12	....	....	....	9.4	....	....	89
July 12	6	1.5	1.7	12.7	83	84	90
Aug. 8	7	.9	1.1	....	87	90	93

Date of test.	Length of time stored under different conditions.	Pelissier wheat. Moisture content (percentage of dry weight). Stored over calcium oxid.	Stored over sulphuric acid (sp. gr. 1.84).	Stored in open vessel.	Germination (per cent). Stored over calcium oxid.	Stored over sulphuric acid (sp. gr. 1.84).	Stored in open vessel.
1917.	*Months.*						
Jan. 12	....	....	....	9.2	....	....	81
July 12	6	1.4	1.8	12.2	58	84	85
Aug. 8	7	1.0	1.1	....	83	86	80

With barley and Sudan grass seed the percentages which germinated in the different tests show only slight irregularities, with no indication of injury from drying, though the moisture content was reduced below 1 per cent.

With Johnson grass seed there was considerable irregular variation in percentage of germination, probably the result of irregularities in temperature control, moisture content of the germinating bed, and length of germination test, with a slight decrease caused by drying. The last tests, made after 10½ months with the seeds containing only 0.1 to 0.2 per cent of moisture, showed no effect of the drying upon percentage of germination. The seeds which did not germinate in 17 days were tested for viability by removing the scales from the caryopses, breaking the seed covering over the embryo, and then incubating the seeds for an additional period of two or three days. These viability tests showed from 90 to 95 per cent to be alive, both of dried lots and of the control lots.

Wheat samples were first taken six months after the beginning of the test. At that time the wheat contained about 1.5 per cent of moisture

and seemed to show considerable reduction in germinating capacity. However, when the next samples were taken a month later, the dried lots germinated as completely as the control lots, although the moisture had fallen to 1 per cent. The unfavorable results of the previous tests must therefore have been due to causes other than previous desiccation. The wheat was not returned to the desiccators after August 8. Subsequent tests made on September 5 and November 25 gave as complete germination for the dried lots as for the control lots.

The degree of desiccation to which all of the seeds, even of wheat, were subjected without injury, is, of course, greatly in excess of any which occur in nature. Wheat, for instance, when stored under laboratory conditions, contains about 8 per cent of moisture in the winter and much more during humid weather in the summer. Wheat as it comes from the field varies widely in moisture content, but apparently is never below 6 per cent, even in the semiarid regions; the minimum for six years according to figures furnished by the Office of Grain Standardization of the Department of Agriculture, was 6.6 per cent.

INFLUENCE OF DRYING UPON RAPIDITY OF GERMINATION AND VIGOR OF SEEDLINGS

The germination of the control lots began somewhat more promptly than the germination of the dried lots, but the differences were scarcely perceptible after the second day of the germination test and were probably due in a large measure to an increase in the time required for imbibition before germination could begin.

Table IV gives additional data from the tests begun on September 5, which are typical of data taken from some of the other tests.

TABLE IV.—*Additional data on germination tests begun on September 5, 1917, after seven months' drying*

Item.	White Smyrna barley.			Svanhals barley.			Kharkof wheat.		
	Calcium oxid.	Sulphuric acid.	Control.	Calcium oxid.	Sulphuric acid.	Control.	Calcium oxid.	Sulphuric acid.	Control.
Percentage germination in 3 days	82	83	83	97	97	97	86	88	90
Percentage germination after third day	2	5	3	0	0	1	6	4	2
Number of coleoptiles emerged in 3 days	6	30	27	14	25	47	......	......	......
Maximum length of coleoptile on third day . . . cm.	0.2	0.9	0.8	0.2	0.8	0.7	0.4	0.4	0.6
Average number of roots on third day	3.2	3.4	3.0	2.8	2.9	3.0	2.1	2.3	2.5
Maximum length of roots on third day, cm.	5.2	4.5	4.2	4.5	5.0	4.0	2.6	2.1	3.1

TABLE IV.—*Additional data on germination tests begun on September 5, 1917, after seven months' drying*—Continued

Item.	Pelissier wheat.			Sudan grass.			Johnson grass.		
	Calcium oxid.	Sulphuric acid.	Control.	Calcium oxid.	Sulphuric acid.	Control.	Calcium oxid.	Sulphuric acid.	Control.
Percentage germination in 3 days	73	76	80	82	88	92	12	19	38
Percentage germination after third day	5	4	4	4	4	0	40	41	40
Number of coleoptiles emerged in 3 days	......	......	......	3	11	157	......	......	......
Maximum length of coleoptile on third day ....cm	0.5	0.4	0.4	0.1	0.1	0.9	......	......	......
Average number of roots on third day	2.0	2.0	1.9	......	......	......	......	......	......
Maximum length of roots on third day, cm	3.2	3.5	4.2	2.1	2.0	3.0	......	......	......

The small number and short length of coleoptiles emerged on the third day from barley samples which had been stored over lime are unusual, as the lots dried over lime did not appear to so poor advantage in any other tests. Except with respect to the development of the coleoptile, little difference appears between the dried lots and the control lots. This difference, however, at least in case of Sudan grass, is not wholly the result of a lower moisture content at the time of beginning the germination tests, as a similar difference appeared in the tests begun on November 25, although with these tests the seeds were left out of the desiccators to absorb water from the air for two days before the germination tests were begun.

The percentages of Johnson grass seeds germinating in three days in case of the tests begun on September 5 showed the effect of the low initial moisture content of the dried lots, but no such difference appeared in the tests begun on November 25 after two days out of the desiccator.

The results outlined in this paper show that all of the seeds used by the present authors, as well as radish seeds as reported by Waggoner, are much more resistant to desiccation than is consistent with Ewart's hypothesis. All of these seeds were dried to 1 per cent of moisture or less without injury; and in the case of Johnson grass seed reduction of the moisture to 0.1 per cent had no injurious effect. Nearly all of the Kentucky bluegrass seeds were still capable of germinating, though with much reduced energy, after the removal of the last trace of water by vacuum desiccation at 100° C. None of the seedlings produced were kept for further growth, but there seems to be no reason to suppose that the dried seeds, except those of bluegrass with the most extreme desiccation, would produce any less vigorous plants than those which were not dried.

SUMMARY

This paper describes experiments to determine the effect on the vitality of certain seeds when dried under varying conditions and for varying lengths of time.

It was found that the percentage of germination was not materially changed when seed of wheat, barley, Sudan grass, Kentucky bluegrass, and Johnson grass was dried to less than 1 per cent of moisture. The percentage of germination of Kentucky bluegrass and Johnson grass seed was not affected when the moisture was further reduced to 0.1 per cent, although the vigor of the Kentucky bluegrass seedlings was greatly reduced. When Kentucky bluegrass seed was further dried in a vacuum oven for six hours at 100° C., the vigor of the seedlings was further reduced, but the percentage of germination was not materially affected. All this controverts Ewart's statements as to the degree of drying which seeds are capable of withstanding and remaining viable, so far as the seeds used in this experiment are concerned.

OCCURRENCE OF COCCIDIODAL GRANULOMA (OIDIOMYCOSIS) IN CATTLE

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OBSERVATIONS ON THE DISEASE IN MAN

Wernicke (*12*) [1] in 1892 discovered the parasite of oidiomycosis in a Brazilian soldier suffering from a peculiar skin affection and described it as a protozoon. Later his pupil Posadas (*7, 8*) made a careful study of the pathologic features of this case, and demonstrated the infectiousness of material from the lesion for several experiment animals. Rixford (*9, 10*), who in 1894 reported a case in a patient living in California, was the first to describe the disease in this country, and two years later Rixford and Gilchrist (*11*) made a further study of the malady, naming the causative organism "*Coccidioides immitis*," believing it to be of protozoan nature. In 1900 Ophüls and Moffit (*6*), having obtained cultures of the parasite, were the first to class it as a mold, and since that year Ophüls (*5*), Wolbach (*13*), MacNeal and Taylor (*4*), and others have established the exact manner of its development as a parasite and on artificial media.

Coccidioidal granuloma in man does not appear to be a widely distributed affection, nearly all of the cases reported being in patients living in the San Joaquin Valley, California. According to Lipsitz (*3*), out of 40 cases reported up to the year 1916, all but 3 were from this locality, and Dickson (*2*) states that 35 of the patients were residents of California and 3 had visited the State. The relatively small number of cases reported is thought by Ophüls (*5*) to be due to the fact that an occasion for infection is very rarely given; others believe that its striking similarity to tuberculosis and certain other diseases causes even the experienced clinician to err sometimes in exact diagnosis.

The disease is observed most frequently in adult males of the laboring class; the primary infection atria being often found in the skin which has been subjected to injury or pricked by some foreign body, possibly a harborer of the specific fungus. The infection appears also to take place primarily by inhalation and possibly by ingestion. However, there still seems to be some doubt as to the manner of transmission of the

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[1] Reference is made by number (italic) to "Literature cited," p. 540.

disease, whether from man to man or whether the parasite passes a stage of its life cycle somewhere in nature and is introduced into the body from this point. Ophüls (*5*) and MacNeal and Taylor (*4*) have expressed the suspicion that the disease is an affection of animals and that through association with such as are diseased man may become infected. In view of the writer's positive findings in cattle, these suppositions are well founded.

Practically all the human cases reported have terminated fatally—Wolbach (*14*) reports a case of recovery—the duration of the disease varying from about three months to nine years. The patients manifest a variety of clinical symptoms which depend upon the organs involved and the extent of the lesions. As the disease progresses from the primary center and becomes generalized, practically all the organs may become the seat of miliary and larger nodules and abscesses, the symptoms corresponding to the location and severity of the lesions. Infection of the bones with purulent osteomyelitis, arthritis, and with compression of the cord and brain is not uncommon. Remittent fever and night sweats appear to be quite constant symptoms, particularly in the later stages of the disease.

DISCOVERY OF THE DISEASE IN CATTLE AND DESCRIPTION OF THE PARASITE

The present writer encountered the infection in bovine bronchial and mediastinal lymph glands forwarded from an abattoir in San Diego, Cal., by Dr. W. M. MacKellar, of Bureau of Animal Industry. The parasite observed in pus from the glands appears to be identical with that found in the lesions of human cases (Pl. 59, A). In the purulent center of the lesion and in the surrounding granulation tissue the parasite is present in considerable numbers. It appears as a spherical body varying from 3 to 35 μ in diameter and having a doubly contoured and highly refractive covering, which is from 1 to 5 μ thick. In some parasites the protoplasm appears very finely granular, while in others it is more coarsely granular and sometimes vacuolated. Large spheres containing many smaller ones of a diameter about 3 to 5 μ are observed, and occasionally one sees these large bodies in a ruptured state releasing the inclosed spores. The broken empty shells may be seen being invaded by leucocytes. Neither mycelia nor gemmation forms are ever found in the lesions, although the latter type is often simulated when two bodies lie in close contact with each other.

LIFE CYCLE OF PARASITE

The process of forming mycelia from the spherical bodies was studied by following the method described by MacNeal and Taylor (*4*). Fresh pus containing spheres was seeded in beef-broth-agar hanging-block preparations and incubated for various lengths of time. After several hours'

incubation microscopic examination of the preparations shows the development of a few short protoplasmic shoots extending out from the capsules of the spherical forms. These growths very shortly assume the character of mycelia, which have a well-defined wall about them, branch extensively, and show septa at intervals. A colony of branched interlacing septate mycelial threads from 2 to 8 μ in diameter is formed about the capsule of the original sphere in the course of 24 hours. Old cultures, particularly those on potato, show an abundance of aerial hyphæ bearing cylindrical or oval conidia which are surrounded by a doubly contoured membrane with a highly refractive and homogeneous protoplasm (Pl. 59, B).

Wolbach (*13*) and MacNeal and Taylor (*4*) have demonstrated the changes that take place in the development of spheres from the mycelial filaments. When rabbits are inoculated intravenously with masses of filaments and their organs are examined histologically at different stages from 24 hours to several weeks, it may be observed that each sphere develops from a segment of mycelium. At the end of 24 hours most of the filaments have broken up into coarse granules and have largely disappeared; a few, however, remain viable and increase in size, breaking up into rectangular segments which continue to enlarge, so that at the end of seven days perfect spheres have formed, some showing endospores.

MacNeal and Taylor (*4*) have demonstrated *in vitro* on special media and under anaerobic conditions morphologic types, including sporulating forms quite similar to the sperical bodies occurring in the tissues.

CULTURAL CHARACTERS

AGAR.—The rate of development at room temperature is rather slow, no growth being visible until after three or four days. Incubated at 37° C., colonies are usually visible within 24 hours. These first appear somewhat circular in outline, of a silvery or grayish color, and very slightly raised above the medium. The mycelia penetrate rather deeply into the substratum, giving the colonies so firm an attachment that in removing some of the growth it is necessary to dig into the medium with a strong platinum wire. After several days the culture assumes a whitish moldy appearance caused by the formation of short aerial hyphæ. In some tubes these occur in abundance, attaining a length of 2 to 3 mm. and spread around on the inner wall of the tubes in profusion, while in others they are much less in evidence. In old cultures the medium shows a brownish discoloration, the growth remaining white. As the agar dries out, the growth assumes a slightly yellowish-brown tinge.

GELATIN.—There is a fairly abundant surface growth similar to that on agar. The aerial hyphæ are not usually so plentiful. In about a week or 10 days a slow stratiform liquefaction begins, and eventually the entire mass of medium is liquefied.

POTATO.—The growth is much more luxuriant on this medium than on agar or gelatin, the development of aerial hyphæ being very marked. The medium becomes brownish in old cultures, and the discoloration is imparted to the culture to a certain extent.

EGG MEDIUM.—The growth is somewhat similar to that on potato, except that it occurs around the margin of the medium and extends on to the sides of the tube for the first week or two; later it spreads over the entire surface of the medium. In the

older cultures the medium becomes dark brown, and after 2 or 3 months the growth is discolored.

COAGULATED COW SERUM.—There is a surface membranous growth with little tendency to form aerial hyphæ. Slow liquefaction of the medium occurs after 2 or 3 weeks.

BOUILLON.—The medium is not rendered cloudy, but a fluffy growth appearing like small pieces of cotton develops in the bottom of the tube. In many tubes a rather firm membrane covers the surface of the medium. Aerial hyphæ do not appear except in old cultures.

MILK.—There is a slow digestion of this medium, three or four weeks being required before there is complete clearing. The reaction remains unchanged. A whitish surface membrane is formed.

Indol is not produced. Dextrose, lactose, and saccharose are not fermented with the production of either alcohol or gas.

THE DISEASE IN CATTLE

Little is known of the disease in cattle resulting from natural infection. The source of infection and the manner of transmission are quite likely the same as in the human cases. However, to judge from the results obtained from experimental inoculations in cattle, these animals are not nearly so susceptible subjects as man.

So far as is known at present, the lesions observed in cattle at the time of slaughter in the abattoir appear to be confined largely to the bronchial and mediastinal lymph glands. These tissues may be the seat of large areas of suppuration or several smaller purulent foci, all of which are usually surrounded by considerable granulation tissue and a fibrous capsule. Upon incising an affected gland there may be squeezed out a thick yellowish and tenacious pus which at once suggests actinomycosis. In fact, the similarity of the lesions produced in the lymph glands by *Coccidioides immitis* and Actinomyces is so striking that the one affection may be easily mistaken for the other upon gross inspection alone. However, microscopic examination of fresh smears of pus at once establishes a diagnosis; in the one case spheres in various stages of development are present in quite large numbers, and in the other the colonies of the ray fungus are detected.

INOCULATIONS OF EXPERIMENTAL ANIMALS

Successful inoculations were made with guinea pigs, rabbits, dogs, cattle, sheep, and swine, the degree of susceptibility in these animals varying in about the order named. Rapid generalization of the disease usually followed intravenous inoculations, and in the guinea pig and dog subcutaneous inoculation proved fatal in a rather brief period. The lesions most frequently encountered are in the form of miliary or submiliary nodules or abscesses involving practically all the internal organs. The histological structure of the nodules is almost identical with that produced by tubercle bacilli—that is, epithelioid cells and a peripheral zone of lymphocytes with giant cells and central caseation. Inclosed in

most of the giant cells one or more of the parasites can usually be seen. Many parasites are also observed lying free in the tissue.

A considerable number of guinea pigs and rabbits were used in the inoculation tests, but a report on a few typical cases will suffice to show the general character of the lesions produced by the fungus recovered from cattle.

GUINEA PIG 1.—On January 19, 1916, guinea pig 1 was injected subcutaneously with 1 cc. of a suspension in normal salt solution of purulent material taken from bovine glands. After about a week a swelling developed at the point of injection, and later there was ulceration of the skin over this area with the formation of a scab. The animal gradually failed and died on April 1 in an emaciated condition. The autopsy revealed the presence of a local ulcer from which a scanty discharge has escaped, matting the surrounding hairs. This lesion is partially scabbed over, and beneath the scab there is considerable thick yellowish pus in which many spheres are found on microscopic examination. On the floor of the sternum there are two rather large grayish nodules. There are several small nodules in the lungs and spleen. Parasites were demonstrated microscopically in teased preparations from these lesions. Cultures of the mold were obtained from the suprasternal nodule.

GUINEA PIG 2.—On April 3, 1916, guinea pig 2 was injected subcutaneously with purulent material from guinea pig 1. It died on June 13. At the autopsy there was observed a local lesion as in the first case; both precrural lymph glands were enlarged and on being sectioned they showed abscess cavities of considerable size containing typical, thick, yellowish pus. On the left side the abscess had broken through the skin. The inguinal, sublumbar, suprasternal, subcostal, and bronchial lymph glands were also involved, all showing suppurating centers of greater or less proportions. Miliary nodules were distributed throughout both spleen and lungs (Pl. 59, C). Parasites were demonstrated microscopically in lesions, and cultures were obtained.

GUINEA PIG 3.—On July 26, 1916, guinea pig 3 was injected intraperitoneally with 1 cc. of a cloudy suspension in a normal salt solution of a 2-month-old culture containing many spores. The animal died on September 3. At the autopsy there was observed a rather large abscess in the folds of the great omentum, marked purulent periorchitis, and uniformly distributed miliary nodules in spleen and lungs. Parasites were demonstrated in both fresh and histological preparations. Cultures were obtained.

RABBIT 1.—On July 26, 1916, 1 cc. of material used in the preceding case was injected into the ear vein of rabbit 1. It died on September 17. At the autopsy there are observed miliary foci in lungs, liver, spleen, and kidneys. Similar lesions are found subpleurally and subperitoneally. Parasites were demonstrated in both fresh and histological preparations (Pl. 60, A). Cultures were obtained.

CALVES 177 AND 184.—On April 3, 1916, calves 177 and 184 were injected subcutaneously on the left side of the neck with 4 cc. of normal salt solution suspension of splenic nodules and purulent material from local lesion of guinea pig 1. In the course of a week both animals developed a local swelling about 75 or 100 mm. in diameter. After several weeks a small ulcer was formed from which a slight amount of discharge oozed, gluing together the hairs below the lesion. The ulcer soon scabbed over, and very shortly the skin showed complete healing.

On September 26 calf 184 was killed, and an autopsy was performed. The carcass was in fair condition, and no lesions except the one at the point of injection were found. On being sectioned the local lesion was found to consist of a rather dense layer of fibrous tissue disposed peripherally inclosing a zone of granulation tissue with a purulent center. Many spherical bodies were demonstrated microscopically in fresh

preparations of purulent material from the lesion, and in histological preparations these forms were observed both inclosed in giant cells and lying free in the granulation tissue (Pl. 60, B, C). Cultures were obtained from this case.

On November 9, 1917, calf 177 was killed and a post-mortem examination made. The carcass was in a very well nourished condition. The lesion at the point of injection had almost disappeared, there remaining only a small indurated tumor under the skin which on being sectioned showed a few yellowish foci containing thick purulent matter surrounded by dense fibrous tissue. Parasites were present in the pus. No other lesions were found.

DOG 258.—On October 19, 1916, dog 258 was injected intravenously with 1 cc. of a cloudy suspension of an old agar culture in normal salt solution. In about a week the animal showed symptoms of dyspnea, which rapidly became very much worse, the dog being found dead on October 29. The autopsy revealed the presence of miliary nodules uniformly distributed throughout both lungs; no other lesions were found. Large numbers of spherical bodies were demonstrated in freshly teased preparations of the nodules. Cultures were obtained.

DOG 249.—On October 19, 1916, dog 249 (much larger than dog 258) was injected intravenously with 2 cc. of the above suspension. This animal developed symptoms similar to dog 258, but slighter later, it appearing to show somewhat greater resistance. Death occurred on November 2. At autopsy lesions similar to those in dog 258 were found. Parasites were demonstrated microscopically. Cultures were obtained.

DOG 326.—On October 19, 1916, dog 326 was injected with 2 cc. of above suspension subcutaneously behind its right shoulder. In the course of a week or two a rather extensive swelling developed at the point of inoculation. The hair came off in a considerable area over the swelling, and an ulcer formed in the skin at this point, which after a time scabbed over. The extension of the disease from the primary lesion progressed gradually, the condition of the animal became steadily worse, and death occurred on December 18. At the autopsy extensive ulceration of the skin and deeper tissues was observed at the point of injection. The dependent subcutaneous and intermuscular tissues showed considerable infiltration with inflammatory exudate. Both prescapular glands were enlarged. The lungs, liver, and kidneys were the seat of miliary nodules. There is a nodule about 12 mm. in diameter present in the suprasternal region. A few parasites were demonstrated microscopically in stained sections of the lung nodules.

SHEEP 559.—On October 19, 1916, sheep 559 was injected with 3 cc. of above suspension intravenously. The animal died on June 17, 1917. At the autopsy the carcass was found in a fairly well nourished condition. The superficial tissues in the region of the left shoulder and the right side of the body from the shoulder to the flank showed a rather marked serosanguineous infiltration resulting from injuries inflicted by cattle kept in the same pen. Both submaxillary, both prescapular, both superficial inguinal, the bronchial, and mediastinal lymph glands had small abscess cavities containing yellowish sticky pus. The lungs were the seat of a severe bronchopneumonia with uniformly distributed miliary nodules and larger caseous encapsulated lesions. There was considerable pleuritis, the visceral pleura being greatly thickened with fibro-plastic exudate and adherent in many places to the parietal pleura. There were many miliary nodules in the liver and a few nodules in the kidneys. The parasite was demonstrated microscopically in fresh preparations from lesions in lymph glands, lung, and liver.

SHEEP 560.—On October 19, 1916, sheep 560 was injected with 5 cc. of the above suspension subcutaneously behind its right shoulder. The animal died on July 14, 1917. At the autopsy the carcass was found to be severely bruised; the local lesion, if any was present, being completely obscured by the extensive bruised condition.

The superficial lymph glands appeared free from infection. The lungs showed numerous small caseo-calcareous nodules, well encapsulated, produced by infestation with *Strongylus ovis pulmonaris*. The bronchial and mediastinal lymph glands were normal. The liver, intestines, and mesenteric glands showed severe infestation with *Esophagostoma columbianum*. Microscopic examination of the various tissues failed to reveal the presence of species of Coccidioides.

CALF 176.—On October 19, 1916, calf 176 was injected with 4 cc. of above suspension intravenously. In about a week the animal had marked symptoms of dyspnea, and appetite began to fail. The condition became rapidly worse, and the calf died on November 1. The autopsy revealed the presence of miliary nodules uniformly distributed in both lungs. The bronchial and mediastinal glands were enlarged. No other lesions were present. The parasite was demonstrated microscopically in teased preparations from the lung nodules.

CALF 181.—On October 19, 1916, calf 181 was injected with 5 cc. of the above suspension subcutaneously on the right side of its neck. The local lesion produced in this case was very similar to that noted for calf 177. The animal was killed on November 9, 1917, and a post-mortem examination made. No abnormalities were found, with the exception of the local lesion, which was essentially the same in character as that reported in calf 177. Parasites were demonstrated in pus from this lesion.

PIG 3059.—On October 19, 1916, the right marginal ear vein of pig 3059 was injected with 2 cc. of the above suspension. After about two weeks small warty growths appeared on the surface of the injected ear, showing first in the immediate vicinity of the marginal vein. Somewhat later this warty appearance was observed over a considerable area of the ear which was markedly enlarged and drooping. The general condition of the animal remained very good. This animal was killed on July 17, 1917, and an autopsy made. The injected ear showed a number of small subcutaneous abscesses located chiefly in the region over the marginal vein. The superficial lymph glands were not involved; nor were the bones. The lungs were the seat of miliary nodules. Numerous small nodules were present in the liver, and there were a few in the spleen. The bronchial, mediastinal, and portal glands showed slight lesions. The parasites were demonstrated microscopically in the pus from the ear lesions and in the lung nodules.

PIG 3053.—On October 19, 1916, the right marginal ear vein of pig (sow) 3053 was injected with 2 cc. of the above suspension. Lesions similar to those described for pig 3059 were also noted in this animal. It was observed that for the first few months following the inoculation the ear lesions gradually became worse. Then there was a considerable period in which little change was apparent, and finally there began a recession of the growths. When the animal was killed, on November 9, 1917, no trace of the ear lesions was found at the autopsy. Complete spontaneous healing had taken place. The lungs, however, were the seat of uniformly distributed nodules. These when examined histologically closely resembled tubercle nodules in structure. Parasites were demonstrated in small numbers in the lesions, some appearing to be undergoing degeneration.

On August 10, 1917, this sow farrowed a litter of four pigs, three of which lived, and were kept with the mother until November 9. On February 20, 1918, these three pigs were slaughtered. The autopsies revealed no lesions in any of the three cases.

ALLERGIC AND SEROLOGICAL TESTS NEGATIVE

With a view to determining whether animals affected with the disease would respond to allergic tests, material for injection was prepared in the following manner: A cloudy suspension in normal salt solution of hyphæ and spores from an old agar culture was autoclaved for 15 minutes

at 15 pounds' pressure and subsequently placed in a shaking apparatus and shaken for three hours. On July 17, 1917, calves 177 and 181 were injected subcutaneously with 5 cc. of this material. The temperatures of the animals prior to injection were normal, and during the next 48 hours neither rise in temperature nor local reaction was noted.

On September 11 the test was repeated, using as injection material 5-cc. doses of a sterilized, filtered, and concentrated (one-tenth original volume) bouillon culture grown for about 6 weeks at 37° C. Negative results were again obtained.

Serums from calves 177 and 181 and from pig 3059 were subjected to both complement fixation and agglutination tests. In the complement-fixation tests two antigens were employed, antigen 1 being the same as the material used in the first allergic test, antigen 2 consisting of some of the substance employed in the second allergic test. No complement-fixing bodies were demonstrated in either case. For agglutination fluid antigen 1 was used. No specific agglutinins were detected.

The negative results obtained in our allergic, complement-fixation, and agglutination tests correspond to those reported by Cooke (*1*), who states that in a human case no specific complement-fixing bodies or agglutinins could be found in the blood serum, using cultures of *Coccidioides immitis* and emulsions of the same organism from human lesions as antigens. He also states that no specific skin reaction could be demonstrated.

CONCLUSIONS

(1) Coccidioidal granuloma (oidiomycosis) has been observed in cattle as a natural infection of the bronchial and mediastinal lymph glands.

(2) The disease is transmissible experimentally to guinea pigs, rabbits, dogs, cattle, sheep, and swine.

(3) Cattle affected with this disease show no response to subcautaneous allergic tests.

(4) Neither specific complement-fixing bodies nor agglutinins are detectable in the serums of affected animals.

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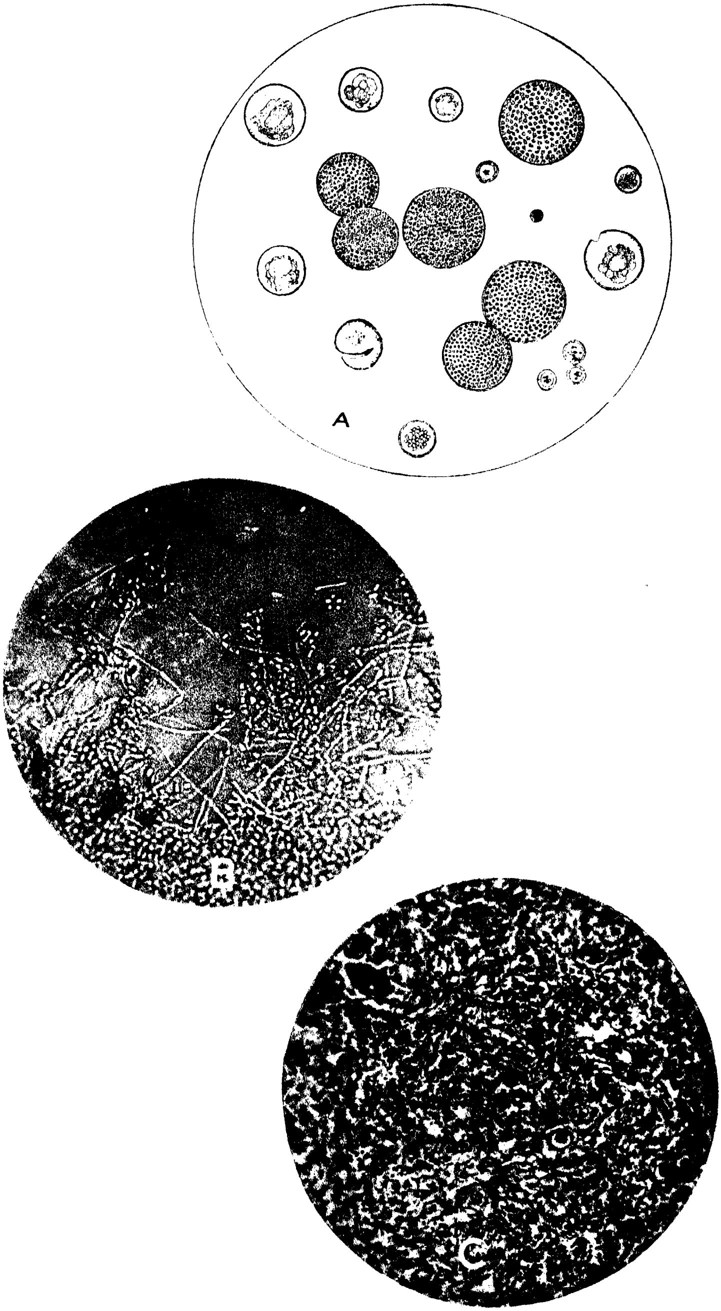
PLATE 59

Coccidioides immitis:

A.—Camera-lucida drawing showing parasites from fresh pus in various stages of development.

B.—Photomicrograph of the hyphæ and spores from an old potato culture. × 220.

C.—Photomicrograph of a nodule of spleen from a guinea pig, showing adult parasites lying free in granulation tissue. × 100.


A
B
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A
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PLATE 60

Coccidioides immitis:

A.—Photomicrograph of the lung of rabbit 1, showing nodules with many parasites. × 100.

B.—Photomicrograph of a local lesion calf 184, showing large giant cells with parasite inclosed in one of them. × 100.

C.—Photomicrograph of a local lesion calf 184, showing a ruptured sporulating form inclosed in large giant cell. × 100.

TISSUE INVASION BY PLASMODIOPHORA BRASSICAE

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INTRODUCTION

Though many workers have studied the clubroot of crucifers, no adequate account has yet been given of the method of infection or of the way in which the parasite becomes distributed in the tissues of its host. The writer has described in detail in a previous publication (*9*)[1] the manner by which a closely related parasite, *Spongospora subterranea* (Wallroth) Johnson, invades the tissues of the potato (*Solanum tuberosum* L.). He has also suggested that a similar kind of infection may hold for *Plasmodiophora brassicae* Wor. and other members of the Plasmodiophoraceae. With this suggestion in mind a study has been made of the clubroot, and it is the chief object of the present paper to give data that seems to make clear the method of tissue invasion.

The occurrence of the parasite within the cells of its host is sufficient proof that it in some way penetrates cell walls. But we would like to know how and when these penetrations take place and the exact method by which the large overgrowth arises. Is the slime mold that produces the great "clubs" with which we are so familiar able to penetrate only the very young rootlets; or is it also capable of attacking older tissues? Does it become distributed by many successive divisions of a few cells originally infected; or is there some other method by which it spreads? Do the many groups of infected cells, the so-called "*Krankheitsherde*," that are distributed throughout the tissues of a single club result from one infection; or is each group the result of a separate infection? What is the relation of the parasite to the host tissues, and by what means does it injure the host plant? These are some of the questions that have not been satisfactorily answered by the students who have already contributed so much to our knowledge of other phases of this interesting disease.

Woronin (*16*) observed amebæ which he believed to belong to *Plasmodiophora brassicae* in the root hairs of young plants and assumed that they would be able to pass deeper into the young root. Favorski (*4*) believes that infection takes place through the ordinary epidermal

[1] Reference is made by number (italic) to "Literature cited," p. 571-572.

cells of very young rootlets rather than through the root hairs. Atkinson (*1*) suggests that the amebæ are capable of—

> streaming out in such fine threads as to enter the roots of the cabbage along with watery solutions of nutrients,

while Eycleshymer (*3*), finding plasmodia in the vessels of fibrovascular bundles, thinks that they may be spread in this manner.

Nawaschin (*13*) observed that infected cells frequently divide and believed that the "*Krankheitsherde*" arise in this way from one or more originally infected cells. He never saw the passage of the parasite from cell to cell and believed that this could not take place after roots begin secondary growth. He says that the tissues probably become infected while very young but that he was unable to observe stages in the process of tissue invasion.

In a recent paper on clubroot Chupp (*2*) takes up the problem of infection and distribution of the organism in the host tissues. Like most other students of the disease, he is of the opinion that only very young rootlets bearing root hairs are susceptible and states that, so far as his observations go—

> there seems to be no question but that penetration does take place through the root hairs and through these only.

He confirms Lutman (*10*) in the observation of actual cell-wall penetrations. In describing the behavior of infection amebæ, Chupp says:

> They may enter in almost a straight path as far as the endodermis,

and argues that a single ameba might give rise to from one to probably six "*Krankheitsherde*" in the primary cortex. He does not describe the infection of the stele, which is obviously the important part of the problem of tissue invasion. Woronin (*16*) long ago pointed out that the primary cortex of young cabbage roots breaks down and is thrown off soon after secondary growth begins. Favorski (*4*) observed that most of the cells infected with Plasmodiophora lie within the central cylinder.

EXPERIMENTAL METHODS

The methods that have been used in each part of this work will be given at the time the different experiments are described. A few points need mention here. Cabbage plants have been used as the host in all cases. The plants were grown in pots in a greenhouse for winter work. In summer they were grown outside in a garden. Most of the microtome sections were cut in thicknesses varying from 5 to 10 μ. A few have been cut 20 μ thick. Several fixatives were used to kill material, but Flemming's solutions proved most satisfactory. All of the sections have been stained with Flemming's triple stain. Nawaschin's (*13*) method of leaving sections unbleached so that the oil in the plasmodia, blackened by the osmic acid of the fixative, may serve to make the parasite stand out sharply from the protoplasm of the host, has been used extensively.

TIME AND NATURE OF INFECTION

It has seemed desirable to study the early stages of infection. This was necessary in order to test the truth of the statement that only very young rootlets are liable to be invaded. We wish to know the exact age at which tissues become immune, whether the parasite can pass through the rootcap and infect the roottip, or whether it passes in through root hairs only. For this work young roots that had been exposed for various periods of time to soil infested with spores of the parasite were fixed in Flemming's weaker solution, embedded in paraffin in the usual way, sectioned on a microtome, and stained with Flemming's triple stain. If the organism enters through root hairs only, it should be possible to find a stage when the root-hair region would be infected and other parts of the young root would still be healthy.

A careful study of many of these sections did not yield the results hoped for. In all cases observed the root was found to be either thoroughly infected or quite free from infection. Stages in the process of infection were not to be observed, and the disease was never confined to any special zone of tissue. One thing that this study did make clear, however, was that the organism sometimes gets into the roottip. Cells in the region of most rapid growth were often found to contain small plasmodia, and the writer was able to confirm the observation made by Nawaschin (*13*) and others that the parasite is distributed to a certain extent by host cell divisions.

Since the stained sections did not show the path of entrance of the organism nor indicate the age of tissue that is attacked, a somewhat different method was resorted to. The soil was carefully washed away from the young roots of some healthy plants. Then a small paper cylinder about 1 cm. long and 0.5 cm. in diameter was slipped over each root. The cylinders were placed at different distances from the roottip and always at a part of the root from which no branches arose. Care was taken not to injure the young roots. They were then filled with moist earth containing spores of *P. brassicae* and the ends were sealed with melted paraffin. After attachment of the cylinders the roots were covered with earth and left for future examination. They were usually examined after a period of approximately two weeks. Infection resulted in practically every instance. That part of the root contained within the cylinder became swollen, no matter whether the cylinder was placed near or far back from the roottip. Those portions of the root outside of the cylinder never showed infection. These experiments proved that the parasite is able to attack tissues far back of the root-hair region, and led to other tests that have yielded interesting results.

It was soon found that the stems of young cabbage plants, as well as the older roots, are susceptible to the disease. The earth was removed from around the stems of plants of different ages growing in pots.

They were then washed clean and a small bit of infectious material was placed on each a short distance below the earth line. Care was always taken to place the infectious material on a portion of the stem that was smooth and free of roots. The inoculum was then sealed to the stem by means of melted paraffin. Sometimes instead of sealing with paraffin it was held in place by wrapping the stem with a small cotton string. Inoculated portions of the stem were wrapped in much the same way that the nurseryman wraps the stems of budded plants. The object was to prevent the inoculum from spreading from the tissue to which it was attached, and both methods served the purpose equally well. After the infectious material was fixed to the stems the earth was replaced and the plants incubated for various periods of time. Plate 61 shows a cabbage plant that was treated in the manner just described. This particular plant was inoculated when about 2 months old. The picture was taken approximately six weeks after inoculation. The small roots coming from the club and from the stem above the club were not present at the time the plant was inoculated. This illustration shows the size of the gall that may result from an original infection of a small circular area of tissue not more than 2 mm. in diameter. It will be seen that only the tissues adjacent to the spot where the infectious material was sealed are diseased. The fibrous roots are all free of disease. Plate 62 shows a portion of two other plants along with the plant shown in Plate 61 for comparison. These two clubs are smoother than that on the plant shown in the middle. This is because fewer branch roots have come from them. The clubs are in general outline spindle-shaped, but they are thicker on one side than on the other. The thick side is the one to which the inoculum was sealed. During the last summer the stems of more than 2,000 plants were inoculated. These plants varied in age from 1 month to more than a year. Without a single exception they became diseased. Old stems an inch or more in diameter became infected almost as readily as young ones.

These experiments bring out two important facts. They show that old tissues are readily penetrated by the parasite and that root hairs are by no means necessary to infection. In the second place they show that the disease spreads from a point of original infection to adjacent tissues.

MORPHOLOGY OF THE CLUB

The most casual observation of the roots of diseased cabbage plants reveals the fact that many of the overgrowths are not the irregular swellings that one might expect if they resulted from a large number of separate infections by freely moving amebæ each independent of all the others. Such a fortuitous method of infection might give tumors of many different sizes and forms, but it would hardly produce the definite

spindle-shaped clubs that are so characteristic of this disease. If a number of clubs are brought together side by side, it will be seen that although they may differ greatly in size many of them are alike in shape. Some typical spindle-shaped clubs are illustrated in Plate 63. The clubs shown in this illustration were not produced artificially, but were taken from plants grown on an infested field. Those produced by artificial infection of a small circular bit of tissue and shown in Plates 61 and 62 are essentially like the ones resulting from natural infection in the field and shown in Plate 63. The one-sided knobs at X in the figure indicate the point of original infection in each case. Each club is a morphological unit and the result of one primary infection. This fact is very important to an understanding of the disease and lies at the basis of the explanation of the morphological changes which occur.

It often happens, however, that several points that are not very distant from each other become infected. In that case the swellings may fuse together in such a way as to give rise to an irregular-shaped growth or compound spindle. Plate 64, A, shows a portion of two swellings that are about to fuse together. During the later stages of the disease branch roots arising from either the simple or compound spindle become swollen and serve to distort the original form. In this way badly diseased specimens often become quite irregular in shape, but even in these one sees that the overgrowth is made up of a large number of tapering elements. The spindle-shaped tumor so characteristic of the disease results from the reaction of the host to the stimulus produced by the parasite as it spreads gradually through the tissues from the point of original infection.

STAGES IN CLUB FORMATION

There are two general methods by which cabbage cells become infected. The first may be designated as the direct method and includes all cases of direct penetration. The second is by host cell divisions. This is an indirect method of cell infection. Both of these methods have been known to earlier workers; but the relative importance of the two methods has not been previously recognized. Distribution by host cell division was observed and described by Nawaschin. It will not be taken up in detail in this paper. The direct method of infection deserves further study.

There are two good ways of determining cell-wall penetrations. One is by actual observation of stages in the passage of the parasite through cell walls. The other is by observing the advance of the plasmodia in successive stages of infection. Actual cell-wall penetration will be described later. Advance of the plasmodia in successive stages of infection will be considered here.

The notion that the amebæ of *Plasmodiophora brassicæ* must enter the host through root hairs has gained a strong foothold among students of

the disease. While Favorski (4) expressed the opinion that infection takes place through ordinary epidermal cells, he, too, believes that only young roots are susceptible. This mistaken notion has been a great hindrance to a correct understanding of the disease. All previous attempts to study early stages of infection have been carried out with young rootlets. The infection is undoubtedly very rapid in such young organs. The cell walls are thin, and the parasite has to pass through only a few layers of cells before it reaches the central portions of the root. In older tissues the penetration is more difficult; the organism must pass through many layers of cells, and the study of its spread from tissue to tissue is much easier to accomplish. For this reason the writer has used rather old cabbage stems in his study of tissue invasion.

The method has been to pull the earth away from the stems of potted plants, place a bit of inoculum on one side of each stem, and then put the earth back in place. Portions of the stems of these plants were then fixed in Flemming's stronger solution at intervals of one day. This fixing began one day after inoculation and continued for three weeks. At the end of this period infection was evident in the plants first inoculated, for the swellings had reached a considerable size. These stems were embedded in paraffin, sectioned, and stained. None of the cells of any of the stems studied became infected during the first eight days after the inoculum was placed on them. Some of the stems showed a few infected cells on the ninth and tenth days. These cells were in the outermost portions of the secondary cortex. No abnormal growth of infected cells or of cells surrounding them could be observed. On the eleventh day a very small swelling was seen on most of the stems, and somewhat deeper layers of cells showed infection. In a number of cases those swellings were so slight that they could not be seen with the naked eye, and it was not known that the overgrowth had started until after stained sections were studied under the microscope. Plate 64, B, shows a section through a swelling on a stem that was fixed 11 days after inoculation. Only the outer layers of the secondary cortex are infected. The parasite has already stimulated these layers, and they are beginning to show abnormal growth. The dark specks that may be seen in the tissue of the protuberance are the young plasmodia. They are as yet very small, usually showing not more than half a dozen nuclei and little of the oil so characteristically present during the period of their vegetative growth. These small plasmodia gradually increase in size as we pass to later stages of infection. This is well shown in the illustrations. It is interesting to note that the nuclei of the host cells and also the nuclei of the cells immediately surrounding the region of infection are more than twice their normal size. Although the inner cortical tissues are still free of infection, the nuclei of the cells in the cambial region beneath the small plug of infected tissue are much larger than normal cambium nuclei. The

cells in this region are also somewhat abnormally enlarged, but their enlargement has not kept pace with the enlargement of the nuclei. These changes in the cabbage cells in advance of infection show that the growth stimulus acts at a considerable distance from infected cells. This suggests that the stimulus may be some substance which diffuses slowly from infected cells into the surrounding tissues. The cells that contain the parasite are, as might be expected, the ones that make most rapid growth.

The infection continues to spread in all directions during the twelfth and thirteenth days. Plate 65, A, shows a section through a stem 13 days after inoculation. The parasite has passed deeper into the host tissues. Some of the plasmodia have already reached the cambium. Infection has also spread to the sides as well as downward, and the plug of diseased tissue is rapidly becoming larger. Nuclear and cell division as well as cell growth is greatly accelerated. The plasmodia have also increased in size and contain more nuclei and much more oil than they did two days earlier. The parasite seems to be a heavy feeder. Not only does it make rapid growth, but it begins to store up oil very soon after entering the host.

Sections through stems 14 and 15 days after infection show a still further advance of the fungus. It is no longer confined to a small volume of tissue and is spreading rapidly in all directions from the original point of infection. Plate 65, B, shows a section through a stem 15 days after inoculation. Some of the plasmodia have passed beneath the cambium layer. Many of the host cells are very much enlarged, especially near the point of original infection. During the sixteenth and seventeenth days the parasite spreads still farther into the healthy tissues of the stem. It has not penetrated very much deeper, however, and does not seem to be able to attack the woody parts, at least not to any very considerable extent. Plate 66, A, represents a section of a stem 17 days after inoculation. Here the parasite may be seen spreading along the cambium. Plate 66, B, shows a section through a stem 19 days after inoculation. It will be seen that the infected cambium has been active and that growth in this region has contributed very materially to the swelling that is taking place. The outer cortical region has also grown until it is now more than twice as thick as it would normally be. Some of the cells in the cortex are greatly enlarged. Those in the cambial region remain small. While the thickening of the cortex is accomplished more by cell growth than by cell multiplication, the swelling in the cambium region is brought about largely by an increase in the number of cells. Plate 67, A, represents a portion of a section through a swelling on a stem 21 days after inoculation. The infected area is now too large to be included in a photograph of reasonable size. The plasmodia in the tissues of this section are much larger than those found in any of the earlier stages. The parasite has also spread

around the stem and the illustration shows plasmodia in the cambium on the side of the stem opposite the point of original infection.

By this time the disease has passed beyond what may be designated as the early stages of infection. We have seen that the parasite passes successively through the outer layers of the secondary cortex, through the phloem region, into the cambium and finally even into the undifferentiated tissues beneath the cambium. We have also seen that it spreads to the sides as well as downward from the point of original infection, so that by the time the cambium is reached this little plug of diseased tissue has become much broader than it was when infection started. Up to this point the invading organism has followed no special course, but has penetrated with almost equal rapidity in all directions through the bark.

After reaching the cambium the plasmodia no longer penetrate the different tissues with equal readiness, but follow what is undoubtedly the path of least resistance. The intruder now becomes what may be termed a "cambial parasite." Its further spread up and down and around the stem is through the cambium and the layers of undifferentiated cells immediately adjacent. These cells are young and rapidly growing; their cellulose walls are still very thin and probably offer little resistance to penetration. It may also be that in this region of most vigorous growth there is a more abundant supply of food materials than in the older tissues. It spreads through the cambium around the stem until it reaches the side of the stem opposite the point where it originally entered the bark. It also spreads up and down the stem, forming a cylinder of infected cambial tissue. The distance that the fungus travels in its spread through the cambium seems to depend largely on the condition of the host, especially as regards age and rate of growth. If the host plant is young and growing vigorously it may infect the cambium for a considerable distance from the point of original penetration. The writer has observed cases where the cambial infection has extended for as much as 6 inches up the stem from the point where it originally entered. Plate 67, B, shows a longitudinal section through a young stem having a diseased cambium. It should be noted that infection has extended far beyond the region of swelling. The plasmodia are either in the cambium cells or in the cells adjacent to it. The cortex is free of infection for a long distance up the stem. Plate 71, A, shows the distribution of young plasmodia in the cambium of an old stem.

One might think that the disease would continue to spread until the cambium of the entire plant would be infected. This, however, is not the case. At first the spread is very rapid, but during the later stages of the disease it becomes slower and slower and finally almost ceases. Although, as above noted, the swelling does not always extend as far as the cambial infection; this infection, nevertheless, determines very largely the length of the spindle. If the cambial cylinder becomes infected for

a considerable distance from the point of entrance, the spindle will be long; if the infected cylinder is short, the spindle will be short.

The mature spindle or club results largely from the abnormal growth of the infected cambium. By direct penetration of the parasite and through the division of infected cells the disease is spread in both directions from the cambium. If the stem is quite old and the phloem elements well differentiated a large part of the spread from the cambium is through repeated divisions of infected cambial cells or by the division of diseased cells that were split off from the cambium after it became infected. A cross section through such an old stem shows the wood and the outer portions of the bark entirely free of infection. Between this wood and outer bark there is a band of infected tissue. This condition is well shown in Plate 68, A. The inner portions of the wood are entirely free of infection, as are also the outer portions of the bark. For the most part only those tissues that have developed since the cambium became infected show the disease. Plate 68, B, shows a portion of a cross section of a somewhat older stem. It will be seen that the layer of noninfected bark is much thicker here than in A. That the infection shown in B is younger than that shown in A is indicated by the size of the plasmodia in the two sections. The band of infected tissue between wood and bark is very definite in each case. Plate 69, A, gives a portion of a longitudinal section through another stem that became infected after its wood and bark elements were well differentiated. Longitudinal sections through different portions of such a stem show that down near the point of original infection the band of diseased tissue is broad, while farther away it is narrower, and finally comes to a point at the place where the cambium is healthy. The plasmodia shown in Plate 69, A, are almost mature, and spore formation is beginning. The infection here is older than that seen in Plate 68, A, and much older than that shown in Plate 68, B. The three figures show three different stages of the development of the disease in old stem tissues.

If the stem is attacked while the plant is young the greater part of its tissues are still very susceptible to infection The course of infection is much the same in these stems as in the older ones, except that here the fungus spreads more readily from the cambium out into the several layers of the bark and in toward the xylem.

Sections through stems have been used to illustrate the above description of infection. It has been easier to obtain the different stages in the stem tissues than in root tissues. This is probably because the infecting plasmodia travel more slowly through the hard stems than through the roots. It has, nevertheless, been possible to observe many of the different stages of infection in roots also. Although the series of stages is less complete for the root, enough of them have been observed to make certain that the general method of invasion is the same in the two organs.

Two schematic drawings have been prepared for the purpose of indicating in a general way the path followed by the infecting plasmodia and the direction of infection in the different tissues (fig. 1, 2). These drawings apply equally well to both root and stem. The arrows are meant to show schematically the general course taken by the parasite as it passes in through the tissues and produces a typical club. (The actual path along which the infecting plasmodia travel can not, of course, be represented by straight lines.) Besides showing direction of infection, the arrows also indicate the extent to which the different tissues become infected. The cambium is represented by lines without arrow heads. The arrow lines running parallel to it indicate in each case the direction of infection.

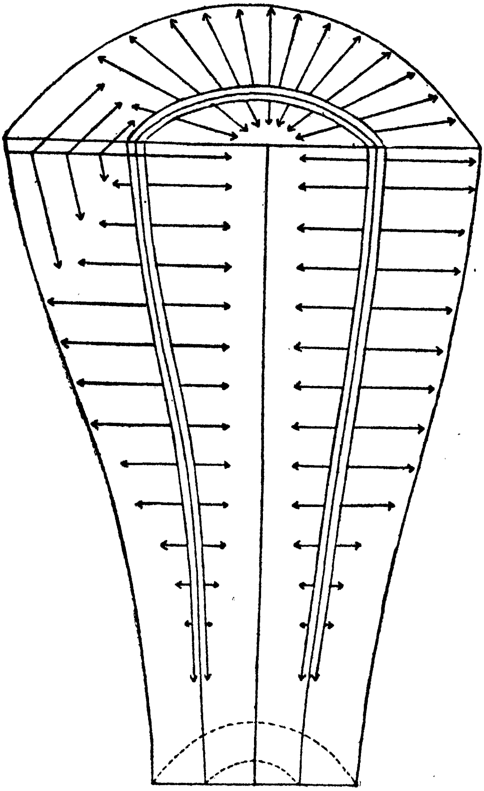

FIG. 1.—Diagram showing the course taken by the infecting plasmodia in a young cabbage root or stem. The arrows indicate the direction and extent of infection.

Each of the two figures represents one half of one end of a club. In order to obtain such a portion the club is cut transversely through its thickest part. Then one of the ends is split longitudinally in half. Figure 1 illustrates the infection of a rather young root or stem, while figure 2 shows the infection of a somewhat older organ. It will be seen that, although the course taken by the parasite in the two cases is the same, the extent of infection is somewhat less in old plants. In the old root or stem the vascular elements are well developed, and the parasite does not penetrate so deep into the tissues on either side of the cambium. In young organs where the vascular elements are not so well developed the plasmodia pass deeper into the tissues on either side of the cambium. The diagrams show the parasite entering the host tissue at the points where the arrow lines pass into the cortex. From this point it spreads to the sides and downward and

finally reaches the cambium. In the cambium it spreads around the stem and also up and down the stem. From the cambium it passes inward toward the pith and outward toward the epidermis. As is shown in the diagrams (fig. 1, 2), most of the cortical tissues become infected from within. This was not to be predicted and is exactly opposite to the direction that previous workers have assumed. The greater part of the cortex becomes diseased through secondary infections rather than by primary infection, as has usually been supposed.

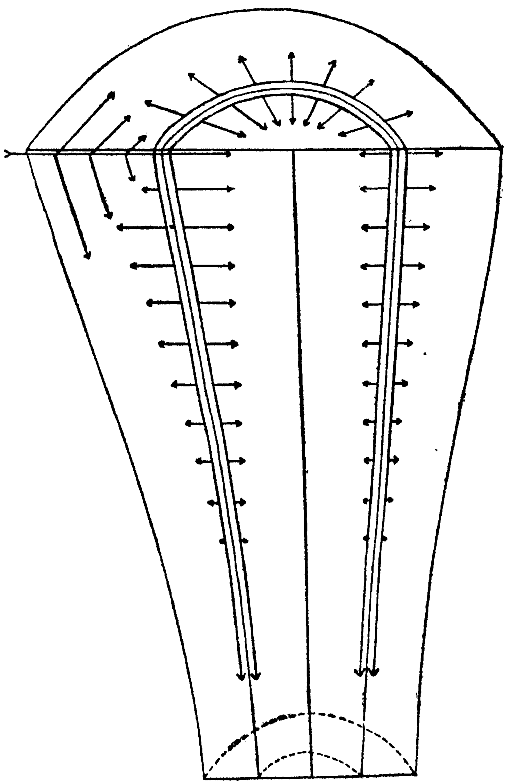

FIG. 2.—Diagram showing the course taken by the infecting plasmodia in cabbage roots or stems that become infected after vascular elements are differentiated. The arrows indicate the direction and extent of infection.

As noted above a few of the outermost cells of the cortex of stems that were fixed nine days after inoculation showed infection. At this time there was no swelling whatever. In no case was an epidermal cell found to be iufected. A few young plasmodia were observed in the second, third, and deeper layers of cells. Usually these plasmodia contained from two to six nuclei, but in a few cases uninucleate ameba were seen. In all probability the primary infections are brought about by such amebæ. The writer has never been fortunate enough, however, to observe them passing through the walls of epidermal cells, and until such observations are made it is not possible to say whether uninucleate or multinucleate bodies are concerned. The fact that epidermal cells are seldom infected permanently probably accounts for the difficulty in observing the parasite in these cells. It is also not known whether the disease starts from one primary infection or from the infection of several adjacent epidermal cells. In any case primary infections are local and are confined to very small areas. One might think that, if the spores of *P. brassicae* are closely packed

around the stem of a cabbage plant, infection would be general over the inoculated surface of the stem. This, however, is not the case. Under such circumstances infection starts at a good many different points, but it is never general over the entire surface. No evidence has been obtained that primary infection takes place through wounds. What it is that determines the original point of entrance is a problem that remains to be solved.

PASSAGE OF PLASMODIA THROUGH HOST TISSUES

As has already been said, both Lutman (*10*) and Chupp (*2*) have described and pictured plasmodia in the act of passing from one cell into another. Neither of these authors has attempted to show the stages by which this process takes place or to describe it in detail. The writer has, therefore, made a further study of this phase of the problem.

In sections of young roots plasmodia were occasionally seen in the act of what seemed to be cell-wall penetration. The process was so seldom observed, however, that the writer was never quite able to convince himself that the appearances met with might not be due to the effect of the fixative on the pathological tissues. In the study of the early stages of infection, cell-wall penetrations were found so frequently as to leave no doubt but that they play a part in normal infection. These penetrations were especially abundant around the edges of the small infection plugs in material that was killed 13 and 14 days after inoculation. Material of this age showed a number of different stages in the process of cell wall penetration. A few instances were observed where plasmodia were closely applied to cell walls but in which no penetration had occurred. Such a case is shown in Plate 70, B. The infected cell is somewhat plasmolyzed by the fixative, but the plasmodium has not been pulled away from the cell wall. The wall, moreover, is seen to bend away from the plasmodium as though a pressure were being exerted on it by the parasite. Plate 70, C, shows another very young plasmodium that has penetrated a cell wall and is beginning to pass through it. This plasmodium contains three nuclei. Figure D shows a little later stage in the passage of a plasmodium through another wall. The parasite has penetrated the wall, but has not yet entered the protoplast of the new host cell. Plasmolysis of the two cells by the fixative has been an advantage in bringing out this fact. The plasmodium contained so much oil that it was not possible to see the nuclei. A still later stage is to be seen in figure E. Here the host cells are not plasmolyzed. The plasmodium is well stained and the opening through which it is passing is clearly shown. Much the same stage is represented in figure F. In this figure the parasite is shown passing through the end of a cell that lies near the cambium. The stage represented in figure G is very interesting because a nucleus is in the act of passing through the opening in the cell wall. This is the only instance in which

such a stage has been found. Here, again, the plasmolysis of the host cells makes the parasite stand out more clearly than it otherwise would. Figure H shows a plasmodium passing through another cell wall. The section in which this plasmodium was found had been left overnight in acid alcohol in order to remove thoroughly the stain from the cell walls. While this was being accomplished, the stain was also removed from the plasmodium to such an extent that its nuclei can no longer be distinguished. Figure I shows a rather large plasmodium in the process of passing through the end of a cell in the region of the cambium.

Late stages in the process of cell-wall penetration have not been found. It may be that these stages require a comparatively short period of time. It may also be that a part of the difficulty lies in distinguishing them from the early stages of penetration because in some cases it is not easy to determine in which direction the plasmodium is passing. The general direction in which migration is taking place helps to determine this point. The bending of the cell wall, where this occurs, is further evidence of the direction of passage. In this way we know that the plasmodium shown in figure I is passing from the cell on the left to the cell on the right. In a number of cases it was observed that the nucleus of the old host cell lies near the plasmodium as it passes out into the new host cell. Figures D, G, and H show such cases. Figure J shows an instance in which the plasmolysis of the host cells seems to have broken a migrating plasmodium into two parts. No hole could be seen in the cell wall, however. A careful study of tissues through which plasmodia are rapidly migrating has been made, but in no case has a hole been found in a cell wall except when a plasmodium is actually passing through the wall. This would seem to indicate that the holes close up soon after the plasmodia pass through. The drawings show that the hole varies considerably in size in the different cases studied.

The migrating plasmodia undoubtedly grow and divide as they pass from cell to cell, but this part of their behavior has not been sufficiently studied up to the present time. In all cases observed the cell out of which the plasmodium is passing is being left free of infection. Such cells act as temporary hosts for the plasmodia that pass through them without leaving any trace of the cells having been infected. While some of the plasmodia move deeper and deeper into the tissues, others remain permanently in certain of the host cells. Here they grow and in time mature spores. The migrating plasmodia are in all cases small. They contain few nuclei and are relatively free of the oil that is so abundant in the larger plasmodia. It is not known what determines whether a plasmodium shall stop in a given cell or shall pass on to some other cell. The writer has observed, however, that the infecting plasmodia in the edges of diseased areas tend to keep a certain distance apart as though they might have a repelling influence on each other. It is not believed

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that plasmodia which have grown to be large and are heavily charged with oil are able to leave the cells in which they live. Such plasmodia have never been found penetrating cell walls.

In the above description of cell-wall penetrations the multinucleate masses of parasitic protoplasm have been called "plasmodia." These masses might be looked on by some as multinucleate amebæ. It is usually considered that plasmodia arise through the fusion of many separate amebæ rather than by the growth of a single ameba into a large multinucleate mass. In the saprophytic Myxomycetes this point does not present difficulties because nuclear division in the ameba is closely followed by cell division. In *P. brassicae* nuclear divisions take place without corresponding divisions of the ameboid protoplast. The result is that uninucleate amebæ may grow into large multinucleate masses which can not be distinguished in any way from plasmodia that arise through fusion. For this reason the writer prefers, for the present at least, to designate all multinucleate masses as plasmodia. It should be kept clearly in mind, however, that the term is used here solely for describing the multinucleate masses seen within the cabbage cells, and does not refer to their mode of origin.

As has already been stated, uninucleate protoplasts have occasionally been observed within some of the outer layers of cortical cells. If no swelling has yet occurred on the stem, it is probable that such amebæ have come directly from spores and represent a very early stage of infection. If considerable swelling has already taken place, it is more probable that they have arisen by division from a plasmodium. These uninucleate masses have been observed in cells of the cambium far removed from the point of original infection. They have also been seen in the growing tip of infected stems. Therefore they do not necessarily represent recent infection. Plate 70, K, L, M, shows small ameboid protoplasts that were observed in cells of the cambium of a plant that had been diseased for approximately six weeks. The one is uninucleate, the other binucleate, and the third contains four nuclei. They are the bodies that continue to spread the disease—namely, the infecting amebæ and plasmodia. Plate 70, N, shows a cambium cell containing several small plasmodia. Three of these are binucleate. The host nucleus is shown in process of division. From the distribution of the plasmodia in the host protoplasm it would seem that cell division might leave four of the plasmodia in one of the daughter cells and only one in the other.

PRODUCTION OF BRANCH ROOTS AND SHOOTS

The distortion of clubs by branch roots has already been mentioned. In fact, the disease seems to stimulate branching. The secondary roots coming from a diseased root may in some instances reach a length of several inches, but they are usually much shorter than this. Sometimes they are so modified that they never get to be anything more than short

knobs. A section through one of these knoblike branch roots is shown in Plate 69, B. When such diseased branches arise from rather young roots, it sometimes happens that the diameter of the branch root is as great or even greater than the diameter of the root from which it arises.

The disease also stimulates the production of buds in places where they do not normally occur. These buds often arise in great numbers from infected roots. This phenomenon was first observed by Woronin (*16*). It has been somewhat more carefully studied by Favorski (*4*).

The tissues of such buds are always infected. In most cases they grow into short fleshy shoots like that shown on a branch root in Plate 71, A. The leaves are thick, distorted, and abnormally succulent. In other cases the buds grow and give rise to sprouts of considerable size. Such sprouts may push above the surface of the ground and become green. An example is shown in Plate 71, B. The leaves of these large sprouts become green and appear quite normal. Sections through such leaves show that the parasite is more or less evenly distributed throughout their tissues. In some cases there is a tendency for the plasmodia to be more abundant in the tissues bordering on the veins. A cross section through a diseased leaf is given in Plate 72, A.

One interesting thing shown by the diseased buds is that they are often unable to respond normally to gravity. Sometimes they arise on the underside of a root and grow directly downward. In other cases they arise laterally and turn downward like the young sprout shown in Plate 72, B. They have also been observed to grow out horizontally, but this is not so common as downward growth.

The writer has studied the diseased buds with a view of determining whether or not the parasite is distributed in them by the numerous cell divisions that occur in the growing tip. This study has consisted in the observations of serial sections through a few of the diseased buds. The observations have not been extended enough to draw final conclusions, but the indications are that the parasite is not distributed to any great extent by cell divisions in the growing tip. Here, as in old roots and stems, its distribution is by means of direct penetration. The plasmodia follow closely behind the growing tip. Sometimes thay even infect some of the cells of this region. Most of the cells, however, have been found to be free of infection in the cases studied. The distribution of the parasite in these sprouts can not be accounted for by growing tip infection as Favorski (*4*) seems to have believed.

HISTOLOGY OF THE CLUB

Some attention should be given here to the response which the host tissues make to the attack of this enemy parasite and more especially to the pathological histology of diseased organs. This phase of the disease has scarcely been mentioned by previous workers. Perhaps the explana-

tion lies in the fact that most investigators have studied only the young rootlets.

As is well known, badly diseased plants wilt during the warmer parts of the day, when transpiration is most rapid. For a certain time at least they recover from the wilting during the night and on cloudy or rainy days. Finally, however, the plants wilt beyond recovery, die, and dry up. The early stages of the disease cause more or less dwarfing, but, so far as the life of the plant is concerned, the critical period is reached when wilting begins. This is the symptom of approaching death.

Most wilt diseases that do not result from an actual destruction of some organ of the plant are caused by vascular parasites. The invading organism gets into the vascular system and interferes with the passage of water from the roots up to the leaves. Several writers have suggested that diseased plants have fewer lateral feeding roots than healthy plants. This is to a certain extent true, but it does not account for the wilting in all cases, since many plants wilt that are abundantly supplied with feeding roots.

The writer has made a study of the vascular elements of infected roots and stems by means of stained serial sections. Small plasmodia have occasionally been found in the tracheids and also in the large vessels. But this is by no means common; it is, in fact, so rare that the possibility of these plasmodia having any appreciable effect on the functioning of the xylem elements is out of the question. Like other dicotyledonous plants, the cabbage during its secondary growth produces, on either side of the cambium, cells that sooner or later become differentiated into xylem and phloem. In the case of the cabbage plant, the differentiation takes place more quickly on the xylem than on the phloem side. The development of xylem tissue thus keeps pace with the increase in transpiration, due to the growth of leaves. When the young root or stem is attacked by the parasite in question the cambium quickly becomes infected, as has been seen. The plasmodia then pass into the layers of undifferentiated cells on either side of the cambium. These cells, instead of developing into vascular elements, as they do in healthy plants, are stimulated to abnormal growth and division. Even the noninfected cells surrounding those that contain the parasite are to a certain degree prevented from developing into vascular tissue. The differentiation of noninfected cells is, however, not entirely prevented, and a small amount of vascular tissue continues to be added to each bundle. The top of the diseased plant keeps on growing—not so fast as in the case of healthy plants—but much too fast for the atrophied development that takes place in the conducting system. In other words, the leaf surface outgrows the conducting system. The parasite may not interfere with the functioning of this system, but it prevents that enlargement which would be necessary to meet the needs of the ever-increasing

number of transpiring cells. The cells of the infected tissues, instead of contributing to water conduction, use up water in their growth. Furthermore, the large swellings themselves, especially when above ground, increase the transpiring surface. Finally on a warm, dry day the critical point is reached. The leaves are no longer able to obtain as much water as they transpire and the plant wilts or, as the gardeners say, "flags."

But not all plants are attacked while still young. It often happens that they escape infection in the seed bed, and contract the disease only after having reached a considerable size. These plants have well-developed vascular systems before they become infected. Can the parasite cause wilting in such plants and, if so, by what means?

In the study of the infection of old stems we have seen that the plasmodia are able to attack the undifferentiated tissues on either side of the cambium, but that it can not penetrate far into the older portions of the bundles. It is unable to attack the woody cells of the xylem, but the undifferentiated cells of the medullary rays are still susceptible. The invader is able to penetrate the rays and to stimulate their cells, so that instead of remaining small and inactive they grow and divide and give rise to a pathological tissue. Whether the medullary cells are penetrated before they begin to grow and divide is not known. The observations of the writer indicate, however, that the growth stimulus travels somewhat in advance of infection.

The growth of the medullary ray cells splits open the woody cylinder. The xylem tissues are forced apart and the bundles distorted in a variety of different ways. Sometimes the splitting up of these tissues is so complete that no two vascular strands can be found near together. In other cases the invasion of the medullary rays is not so complete, and the splitting of the woody cylinder is only partial. It frequently happens that the wood is split into two approximately equal halves.

Plate 73, A, shows a large woody cylinder that is beginning to split apart. The parasite may be seen in some of the cells of the ray that has been stimulated most. Plate 73, B, shows the center of another rather old root. A wedge of diseased tissue has forced apart the two halves. Plate 74, A, shows a further development of the diseased ray. Here the split is complete, and the two halves of the woody cylinder are being forced apart. It will be seen that the right half of the cylinder is beginning to split up into still smaller parts. Plate 74, B, shows a wedge that has grown faster in the center than toward the two edges. It should be noted that the cells composing the wedge are much larger than the cells of the uninfected medullary ray. This is true of noninfected, as well as infected cells. Plate 75, A, shows the woody tissues separated still farther from each other. It is interesting to note that growth is quite uniform in different parts of this diseased ray. The edges of the rays remain almost parallel to each other. Figure B of this

plate shows the halves of another woody cylinder that are being forced farther and farther apart. A longitudinal view of one of these wedges is given in Plate 76, A.

The above examples of ray infection have in most cases shown the wood split into two approximately equal halves. Such cases are easier to show in a photograph than where the splitting is more complete. Plate 77, A, shows a portion of a woody cylinder that is being much more thoroughly split up by medullary infections. By further growth of the infected medullary rays the bundles become more and more separated from each other. Through unevenness in the growth of different portions of diseased tissues they become twisted and distorted in various ways. Sometimes the twisting is so great that a portion of the bundle that was originally nearest the center of the woody cylinder is turned toward the surface of the club, while the portion originally adjacent to the cortex is turned toward the center of the root.

It is interesting to note the further development of the bundles when they are separated from each other in this way. No longer held together in a compact mass, they broaden out so that in cross section they appear fan-shaped rather than wedge-shaped. Figure B of Plate 77 shows a bundle that is beginning to broaden out in this way. Plate 78, A, shows another strand in which the spreading has progressed until the bundle is semicircular in cross section. Figure B of this plate gives a view of a still later development. Here the bundle has grown until it is almost cylindrical and is being further split up by later infections.

The vascular strands, separated from each other and distorted in various ways, are apparently no longer able to function normally. The diseased tissues that separate them probably use up a large part of the water which they transport. In this way the parasite is not only capable of hindering the further development of the conducting system but is also able, by means of medullary infection, to interfere with the functioning of those elements that are already present when it makes its attack. If roots and stems become infected while still young, the injury to their vascular systems consists in hypoplasia of cell differentiation. This also occurs when old organs are attacked, but here, in addition to arresting the development of new xylem and phloem elements, the vascular tissues already present are torn apart through hyperplasia in the medullary rays. For this reason late infection does not prevent the disease from causing the host plant to wilt.

In the above paragraphs emphasis has been placed on the changes wrought by the parasite in the vascular tissues because these changes are so striking and conspicuous. It would be wrong to suppose, however, that these are the only factors concerned in bringing about death or even in producing wilt. The plasmodia in all probability give out substances deleterious to the plant. The yellowing of the leaves during

late stages of the disease suggests the presence of such substances. Moreover, the large galls draw heavily on the food supply of the plant and must lead to profound changes in the metabolic processes going on in the cells of the different tissues.

If roots are attacked while comparatively young, the medullary rays do not for some reason become infected and the woody cylinder remains intact. Most of the abnormal growth that takes place in these roots occurs in the region of the cambium and in the cortex. If, on the other hand, infection takes place after the root is quite old and considerable wood has been produced, the medullary rays, as has been seen, become diseased. The infected rays grow very rapidly, and in many cases give rise to most of the tissues that compose the clubs. The greater part of these tissues are parenchymatous, but weak vascular elements may also develop in them. Such elements are shown in Plates 73, B, and 74, B, and especially in Plate 75, B. They develop in the ray tissues between the bundles and probably aid in transporting water and food to the diseased medullary cells. The long axis of the cells in the tissues between the older portions of the wood are parallel to the direction of growth, while the long axes of the cells between the younger portions of the wood are at right angles to the direction of growth. This is very interesting, since all of these cells arise from the same medullary ray.

The infection of old roots and stems logically falls into four parts, as follows: (1) Infection of the cortex from without (primary infection); (2) infection of the cambium in all directions from the point or points of original penetration; (3) infection of the undifferentiated cells on either side of the cambium and of the inner cortex; and finally (4) infection of the medullary rays. No better proof for direct penetration of tissues by the plasmodia could be wished for than that given by the different stages of medullary infection. It will be seen that direct penetration of the tissues by plasmodia plays a much larger rôle than has previously been supposed.

Former students of the disease seem to have had very hazy notions as to the way in which the galls arise. This is strikingly brought out by Küster's (*8*) reference to gall mother cells. He seems to believe that the cecidia caused by Myxomycetes may result entirely from successive divisions of a single infected cell. We know that individual "*Krankheitsherde*" increase in size through host cell divisions, but these divisions have a small part in distributing the parasite throughout the tissues.

INFECTION OF YOUNG ROOTS

The writer has not made a detailed study of the infection of very young rootlets. He has no doubt, however, that they become diseased through direct penetration. Even in such young tissues host-cell divisions probably play a minor part in the distribution of the parasite.

Before leaving this subject some mention should be made of the root hair infections observed by Woronin (*16*) and others. The writer has also observed and studied these infections. They are often very abundant and conspicuous. It is not surprising that they should have impressed students of clubroot. The writer has never been able to bring proof, however, that a single one of these infections is caused by *P. brassicae*. In every case where it has been possible to follow the further development of the amebæ and plasmodia observed in the root hairs they have been shown to belong to another organism. Two species of Olpidium are known to infect the root hairs of cabbage plants in Europe. One of these, *Olpidium brassicae*, was observed and described by Woronin (*16*). The other, *O. borzii*, has been studied by Nemec (*14*). The writer has found both of these species on cabbage roots in this country. It is, in fact, difficult to find a cabbage plant entirely free from *O. brassicae*. *O. borzii* is less common, but is by no means rare. Both species have been observed to produce long tubes out of which the zoospores pass.

The writer has grown many cabbage plants in soil free of *P. brassicae*, and has observed in these plants all the different stages of root-hair infection that one finds on the roots of plants grown in infected soil. The living plasmodia in the root hairs closely resemble those of *P. brassicae*. But when killed with Flemming's weaker solution and stained with Flemming's triple stain, they show certain characteristic differences that distinguish them from *P. brassicae*. The distribution of the nuclei in these plasmodia is more regular than in *P. brassicae*, and the structure of the cytoplasm is also different. Plate 76, B, shows a cross section of a young root infected with *Olpidium brassicae*. The dark round bodies are the plasmodia. This parasite gets into the root hairs and into other cells in all parts of the primary cortex. It sometimes occurs sparingly also in the outer cells of the secondary cortex. *P. brassicae* is seldom found in abundance in the cells of the primary cortex or in the outer cells of the secondary cortex. It parasitizes the central cylinder and the inner portions of the secondary cortex. *O. brassicae* is never found in these tissues, so far as the writer has observed. The fields occupied by the two parasites overlap somewhat, but for the most part they are distinct.

There seems to be no good reason why *P. brassicae* should not get into root hairs and other epidermal cells of the primary cortex. No doubt it does pass through these cells on its way to the central cylinder. The writer is forced to conclude, however, that the root hairs are of no importance to *P. brassicae* and that Woronin was in error in believing that the organism he observed in root hairs belonged to this parasite. The same conclusion has already been reached by Favorski (*4*), who made a careful study of the root-hair infections and had at his disposal the slides made by Woronin.

RELATION OF THE PARASITE TO THE HOST TISSUES

Several previous investigators have given a certain amount of attention to a study of the relation of the parasite to the host cell in which it lives. From this work we know that the host cell is stimulated to abnormal growth and division, that its cytoplasm becomes vacuolate, that its nucleus enlarges and becomes malformed. We also know that this nucleus divides mitotically and that, on the whole, the cell lives and functions more or less normally.

Previous workers seem not to have studied the relation of the parasite to the noninfected cells of the club and to the diseased tissues as a whole. They have looked upon the individual infected cells and groups of cells as separate and distinct pathological units, each independent of all the others. This was, of course, natural so long as it was believed that each diseased cell or group of cells was the result of a separate infection. As soon as it was recognized that the typical club is a pathological and morphological unit and usually the result of a single infection, the question of the relation of the parasite to the tissues as a whole at once presented itself. It has been shown that the organism travels with great readiness through the cabbage tissues. Why is it, then, that some of the cells in each of these tissues always escape infection? Is there any numerical relation between infected and noninfected cells in different clubs and in clubs from different plants?

In order to study these problems, the writer selected at ramdom 60 diseased plants growing in a field near Arlington, Va. The plants were inoculated on July 16 and were taken for study on September 14, just 60 days after inoculation. They were all approximately 3 months old when inoculated and were in a vigorous growing condition. Small portions of tissue were cut from one or more of the clubs of each of the 60 plants. These blocks of tissue were fixed in Flemming's stronger solution, embedded in paraffin, sectioned on a microtome, and stained with the triple stain. They were usually cut so as to obtain a cross section of the club, but longitudinal sections were also made. Sections from each of these plants have been observed under the microscope and have furnished material for a study of the distribution of the parasite in the host tissues.

As might be expected, a good many different stages of the disease were obtained. In some of the clubs the plasmodia were still rather small. In others they were much larger and in still others spore formtion had taken place. No very early stages of the disease were to be found in any of this material. The organism was found to be irregularly but rather evenly distributed throughout the tissues of all the different clubs from all the different plants. Only in rare instances was there to be found large numbers of infected cells adjacent to each other. Many of the cells of every club are free from infection. The diseased

cells are distributed singly or in little groups throughout the matrix of noninfected cells. These groups, the so-called "*Krankheitsherde*," are usually entirely separated from each other by noninfected cells. They may be either large or small, and the cells that compose them may vary greatly in size.

As sections from the different clubs were studied it became increasingly evident that, in spite of the irregularity in the distribution of the parasite, a rather definite relation exists between infected and noninfected cells. If few cells are infected, the plasmodia become large. If many cells are infected, they remain relatively small. Thirty-two of the plants studied showed the fungus in the spore stage. This is the final stage of the disease and, therefore, the one best suited to a study of the relation of the parasite to the tissues as a whole. The observations of sections from each of the 32 different plants showed that the ratio between infected and noninfected cells varies considerably in the different clubs. The relation between the volume of the spore masses and the volume of the host tissues in which they are embedded seemed much more constant. A detailed study has been made of this phase of the relation of parasite to host.

The method used to determine the volume of spores in a given volume of tissue was the following. The sections from each club were observed under the microscope with low-power magnification, and a careful selection was made of a field that seemed to show an average quantity of spores for the tissues of that particular club. The section was then photographed, and a circular picture was obtained of the field chosen. The parts showing spores were then carefully cut out of each photograph. This operation gave many small bits of photographic paper, each showing a picture of one or more spore masses. The remaining portions of the photograph showed only noninfected cells and those parts of infected cells that were free of spores. In this way the photograph was separated into two parts. One part was made up of small bits showing spore masses; the other of small pieces showing cabbage cells only. The two portions were then carefully weighed on a balance and the weights obtained gave the ratio between the area covered by spores and the area showing no spores. It was found that although different sheets of photographic paper vary considerably in thickness, the thickness of different portions of a given sheet is fairly constant. Since the spore masses are shown scattered about over the picture, any slight variation in the thickness of the paper is not a source of much error. The greatest source of error in this method comes from the difficulty of following exactly the outline of the spore masses when cutting them out of the picture with scissors. By careful cutting, this error remains small, and it is believed that the method has given an accurate ratio between the area of the photograph showing spore masses and the area free of spore masses. This ratio has been determined for

each of the 32 plants studied, and the area covered by spores is expressed in Table I as percentage of total area.

Three of the pictures are reproduced in Plates 79, A, B, and 80, A. The spores cover 30.9 per cent of the surface of the photograph shown in Plate 79, A, 28.8 per cent of that shown in Plate 79, B, and 28.8 per cent of that shown in Plate 80, A. It should be noted that the shape and size of the infected cells vary considerably in the three illustrations. Figure B of Plate 79 shows fewer infected cells than either of the other figures. The infected cells are so large, however, that their spore masses occupy a space almost as great as that occupied by the spore masses shown in Plate 79, A. This figure shows a large number of infected cells, but these cells are small and the total spore mass is only slightly greater than in the two other tissues. In spite of differences in the distribution of the parasite, number of cells infected, size and shape of spore masses, and in spite of the differences in size and shape of non-infected cells, each of the three tissues contain approximately the same quantity of spores. The relation between quantity of spores and host tissues was found to hold with remarkable constancy in each of the 32 clubs studied. This is well shown by Table I.

TABLE I.—*Quantity of spores of Plasmodiophora brassicae contained in average sections taken from 32 different cabbage plants*

Plant No.	Weight of photographic paper—		Part of photograph occupied by spore masses.	Plant No.	Weight of photographic paper—		Part of photograph occupied by spore masses.
	Showing spore masses.	Showing no spore masses.			Showing spore masses.	Showing no spore masses.	
	Gm.	*Gm.*	*Per cent.*		*Gm.*	*Gm.*	*Per cent.*
1	0.272	0.598	31.2	18	0.198	0.759	20.6
2	.236	.642	26.8	19	.256	.743	25.6
3	.239	.645	27.0	20	.298	.665	30.9
4	.281	.601	31.8	21	.268	.551	32.7
5	.271	.592	31.4	22	.286	.516	35.6
6	.219	.619	26.1	23	.230	.607	27.4
7	.199	.647	23.5	24	.194	.643	23.1
8	.180	.631	22.1	25	.250	.557	30.9
9	.179	.663	21.2	26	.318	.520	37.9
10	.332	.574	36.6	27	.255	.580	30.5
11	.220	.587	27.2	28	.206	.605	25.4
12	.241	.615	28.1	29	.235	.612	27.7
13	.235	.667	26.0	30	.239	.590	28.8
14	.256	.610	29.5	31	.175	.677	20.5
15	.280	.690	28.8	32	.245	.675	26.6
16	.239	.743	24.3				
17	.211	.774	21.4	Average	....	....	27.7

If the areas shown in the photographs be considered as of unit thickness then the percentages given in the table represent the space occupied by the spores of the parasite in each of the tissues studied. It will be seen that the quantity of spores per unit volume varies somewhat in the

clubs of the different plants. In a few cases the spores occupy only about 20 per cent of the volume of the club, while in other instances they occupy a little more than 35 per cent of the total volume. On the whole, however, the quantity of spores contained in a given volume of diseased tissue is remarkably constant for the different plants studied. The average volume occupied by the spores in the diseased tissues of the different plants amounts to about 28 per cent. This means that approximately 28 unit volumes are occupied by the parasite in every 100 unit volumes of diseased tissue. In other words, the volume relation between parasite protoplasm and host protoplasm may be expressed by the ratio 28 to 72. The writer believes that in this ratio we have expressed numerically the balance which here exists between host and parasite. This balance is not between the individual host cell and the individual plasmodium within it, but between all of the plasmodia and all of the cells of the diseased tissue. The growth of the plasmodia in a given club is determined by the amount of tissue involved. Each club is as thoroughly diseased as is possible. The noninfected cells are apparently free of infection, not because they have accidentally escaped, but because of some influence which the host exercises over the parasite. There is a limit beyond which the parasite can not go in its growth in the cabbage tissues. How this limit is maintained is a problem that remains to be solved. It may be that the spread and growth of the parasite is held in check through the development of some protective substance in the host cells. The infected cells seem to have some means of controlling the growth of the plasmodia which they contain. If we assume that this control is exercised through the production of a protective substance or antitoxin, then it is easy to suppose that this substance might diffuse out into surrounding cells and thus render them immune to attack. Before the parasite would be able to establish itself again in a cell it would be necessary for it to pass beyond the region of immune cells. Such a theory would account for the distribution of the organism and for the balance maintained between it and the host tissues. Acquired immunity has not been observed in plants. Perhaps this is because plants do not have a circulatory system comparable to animals and because the protective substance, if such is developed, is not carried to parts distant from the foci of infection.

Although the relation between quantity of spores and volume of host tissue is very constant, the relation between infected and noninfected cells varies greatly. The fact has been emphasized that many of the cells of most clubs remain free from infection. However, in rare instances, almost all the cells of a club may show infection. It is interesting to note that in such cases the plasmodia in most of the cells remain very small. These plasmodia do not grow and never give rise to large numbers of spores. They also fail to stimulate the host cells to abnormal growth.

An example of this kind of infection is shown in Plate 80, B. Ordinarily the small cells between the groups of enlarged cells are free of the parasite. In this case, however, practically all of these small cells contain small plasmodium. The difference between this and the usual distribution of the disease is strikingly brought out by comparing the two tissues shown in Plate 80. It might seem at first thought that such a tissue as that pictured in figure B would disprove the theory that cells immediately surrounding those that first become infected are rendered immune. The figure shows that these cells are not always immune to attack. But the failure of the plasmodia to develop normally is the best indication of the influence the large plasmodia exert on surrounding cells.

A COMPARISON OF THE GALLS OF PLASMODIOPHORA BRASSICAE WITH THOSE OF SPONGOSPORA SUBTERRANEA

From the above account of the pathology of *P. brassicae* it will be seen that the disease caused by this parasite differs very materially from that caused by *Spongospora subterranea* (9).

The typical overgrowth caused by *P. brassicae* is spindle-shaped, thick in the middle and tapering gradually toward either end. The overgrowth caused by *Spongospora subterranea* is a knot that protrudes abruptly out of the tissues from which it arises. The nature of the galls produced by these two parasites is in each case the result of a special method of infection. A definite number of cells adjacent to each other are invaded by the plasmodium of *S. subterranea*. The size of the gall produced by this infection depends on (1) the number of cells that are originally infected, and (2) the number of times these cells divide and the size to which they grow. In this way the size of the gall is limited, and it always remains small. It has never been observed that this parasite is able to pass from an infected to a noninfected cell, and there is no indication either direct or indirect that this can occur. It does not spread through the tissues except by means of the large infecting plasmodium. All of the cells of the sorus of *S. subterranea* are infected, and outside of this sorus all of the cells are healthy—that is to say, there is a very definite line between infected and noninfected tissue. If one were to cut out all of the diseased cells in a typical gall of *S. subterranea* he would get only one piece of tissue. This tissue might be called a large "*Krankheitsherde.*" On the root and stem of the potato the gall is almost always on one side only; it never becomes a spindle-shaped swelling, but breaks through the epidermis, and exposes the rough surfaces of the infected tissue.

P. brassicae behaves very differently in this regard. One or more small bodies of parasitic protoplasm enter the host tissue at some point. These bodies then grow and divide repeatedly. They enter into and pass through the living cells. Some of them become established here and there within the cells of the tissue, while others continue to penetrate

farther and farther into the organ that is being attacked. The period of infection is indefinite; it continues up to the time the host plant dies or stops growing. The infected cells of the gall do not lie adjacent to each other, but are distributed in small groups throughout the diseased tissues. If one were to cut out of a typical gall all of the groups of diseased cells, he would get thousands of distinct and separate pieces of diseased tissue. Instead of one large "*Krankheitsherde*" there are many small ones. The mature gall is seldom or never wholly on one side of root or stem. Usually it surrounds the stem. From the very nature of the infection it is easy to see that *P. brassicae* is a much more dangerous disease than *S. subterranea*. If the writer knew absolutely nothing of the damage done by these two parasites, but had before him the account of the way in which each infects its host tissue, he would be able to predict that the one is a much more serious disease than the other. In the one case the disease spreads indefinitely; in the other it does not. The tumor produced by *P. brassicae* is malignant; that produced by *S. subterranea* is benign.

OTHER GENERA OF THE PLASMODIOPHORACEAE

We have seen that the method of infection for *P. brassicae* is very different from that found for *S. subterranea*. This raises the question of how other genera of the Plasmodiophoraceae accomplish the infection of host tissues. In order to answer finally the question, it will be necessary to make a careful study of the method of infection for each genus. This the writer has not done. It may, nevertheless, be of interest in the light of what we now know regarding the nature of the galls caused by *P. brassicae* and *S. subterranea* to compare these overgrowths with those caused by some of the parasites in related genera.

SOROSPHAERA VERONICA.—This parasite causes swellings on the aboveground portions of a number of species of Veronica. The galls have been described in detail by Lagerheim and are pictured by Winge (*15*). They are elongated, more or less tapering swellings which resemble in general outline the galls of *P. brassicae*. Stained sections show that these galls are like those of the clubroot in a number of important respects. The outer layers of the cortex are usually free of the parasite. This suggests that infection may proceed from within. The apparent age of the plasmodia in different parts of the tissues also points in the same direction. In one important respect the distribution differs from that of *P. brassicae*. Instead of it being confined to small groups of cells scattered about through noninfected tissues it invades large numbers of cells that are adjacent to each other. On the whole the galls caused by this parasite are so much like those caused by *P. brassicae* that the writer feels justified in predicting that when the method of infection is worked out for *Sorosphaera veronica*, it will be found to resemble closely that described for clubroot.

SORODISCUS CALLITRICHIS.—This parasite produces swellings on *Callitrichis autumnalis* and *C. vernalis*. The galls are round or somewhat elongated. They are usually smaller than those of *Sorosphaera veronica.* The distribution of the casual organism in small groups of cells scattered about in the tissues of the inner cortex (7) shows a close resemblance to the distribution above described for *P. brassicae*. Here, again, infection has spread to all sides of the stem. The round or slightly elongated gall may be looked on as a short spindle, and might be caused by an infection similar to that produced by the clubroot organism, provided the parasite travels very slowly through the tissues.

TETRAMYXA PARASITICA.—Goebel (*6*) has described and pictured the galls of this parasite on *Ruppia rostellata* Koch. They occur on both stems and leaves and are small and round in shape. It is not possible to determine from his description or his pictures just what tissues are involved. The statement that the region of infection is surrounded by noninfected cortical tissues is very suggestive. What seems to be another species of Tetramyxa has been described by Molliard (*12*) on *Triglochin palustre* L. This species also parasitizes *T. maritinum* L., and has been studied by Maire and Tison (*11*). These authors picture cross and longitudinal sections through the galls. The distribution of the parasite in the tissues is strikingly like that of *P. brassicae.* Maire and Tison have described this fungus as a new genus, Molliardia, differing from Tetramyxa in that it does not form spores. But the failure to observe spores is no proof that spores do not occur, and the writer agrees with Winge (*15*) that, for the present at least, it should be left in the genus Tetramyxa.

OSTENFELDIELLA DIPLANTHERAE.—This parasite which was described and named by Ferdinandsen and Winge (5) causes swellings on the stems of *Diplanthera wrightii* Aschers. The galls produced suggest a method of infection similar to that found for *P. brassicae.* The swelling extends around the stem and the parasite is confined to the cells of the inner cortex. In the upper and youngest part of the overgrowth only uninucleate amebae are present, while in the more mature portions of the gall plasmodia containing many nuclei are found. This suggests that the disease spreads up the stem.

LIGNIERA GRAMINIS.—Two species are included under the genus Ligniera: *Ligniera graminis* (Schwartz) Winge and *Ligniera junci* (Schwartz) Maire and Tison. The genus differs markedly from other genera of the Plasmodiophoraceae in that it does not produce galls. But this failure to stimulate abnormal growth in host tissues is probably connected with the nature of the tissues infected. Only cortical cells are attacked. In all the other genera deeper and more actively growing cells are infected.

From the above discussion it will be seen that the galls produced by *Plasmodiophora brassicae, Sorosphaera veronica, Sorodiscus callitrichis,*

and *Tetramyxa triglochin* are morphologically much alike. In each case the greater portion of the infection is within the central cylinder. The cortex, or at least the outer part of it, is mostly free from disease. The points of similarity suggest that the method of infection may be the same for all of these parasites. The galls caused by *Spongospora subterranea* on the potato differ from those caused by each of the abovementioned parasites on their respective hosts. This difference is probably correlated with differences in method of host tissue infection.

SUMMARY

(1) Cabbage plants of all ages up to one year are susceptible to clubroot.

(2) Old plants are almost as susceptible as young ones, provided they are growing.

(3) The typical club is a morphological unit and is usually the result of a single primary infection.

(4) Sometimes the swellings resulting from two or more primary infections may fuse together to produce a compound club. Such clubs are more irregular in outline and often have greater length than those resulting from a single primary infection.

(5) The spread of the disease from points of primary infection is accomplished through direct penetration of cells by infecting plasmodia.

(6) Host cell divisions increase the number of infected cells but have a very small part in distributing the parasite throughout the tissues.

(7) Infection by direct penetration may be divided into four different parts as follows: (1) primary infection of cortical tissues and penetration to the cambium; (2) infection of the cambium in all directions from the point of original penetration; (3) passage of the plasmodia out from the cambium into the cortex and in from the cambium toward the xylem region; and (4) infection of medullary rays.

(8) The infection of a given cell may be either permanent or temporary. If it is temporary, it has no noticeable effect on the cell. If permanent, it stimulates the cell to abnormal growth and division.

(9) The growth stimulus is diffuse—that is, it acts on the noninfected cells of diseased tissues as well as on the infected ones.

(10) The stimulus seems to travel in advance of infection. This is easiest to observe in the early stages of infection and in the infection of medullary rays.

(11) The disease stimulates the production of branch roots and shoots. These branches become infected by direct penetration of plasmodia from the diseased tissues out of which they arise.

(12) Diseased shoots are frequently unable to react normally to gravity, as is shown by their horizontal or downward growth.

(13) A single infection may give rise to many thousands of separate and distinct "*Krankheitsherde*."

(14) The mass of parasitic protoplasm in a given volume of diseased tissue is remarkably constant in different clubs and in the clubs of different plants.

(15) The average volume relation between host and parasite in the tissues studied is approximately given by the ratio 28 to 72. This is a numerical expression of the balance that exists between host and parasite. A unit volume of diseased tissue always yields approximately the same quantity of spores.

(16) It is suggested that noninfected cells in diseased tissues may be immune.

(17) The wilting of diseased plants is in part due to hypoplasia of cell differentiation in the xylem portions of bundles and to the splitting up of the woody cylinder through the infection and growth of the medullary rays.

(18) The method by which *Plasmodiophora brassicae* infects host tissues differs markedly from that of *Spongospora subterranea.*

(19) If we judge by the kind of galls produced and by the position of diseased tissues it would seem that the method of infection for *Sorosphaera veronica*, *Sorodiscus callitrichis*, and *Tetramyxa palustre* may be similar to that found for *P. brassicae.*

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PLATE 61

Plasmodiophora brassicae:

A cabbage plant 3½ months old; photographed six weeks after being inoculated at a single point on one side of the stem.

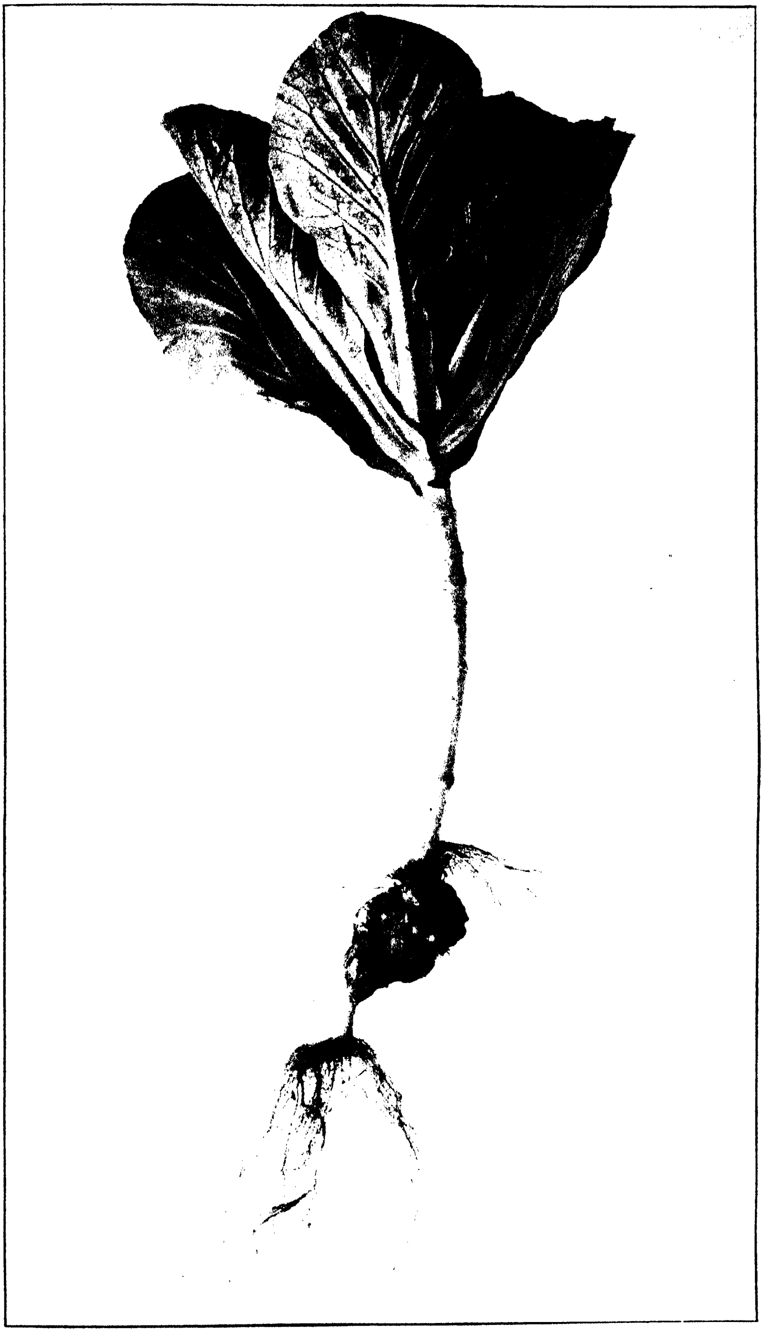


PLATE 62

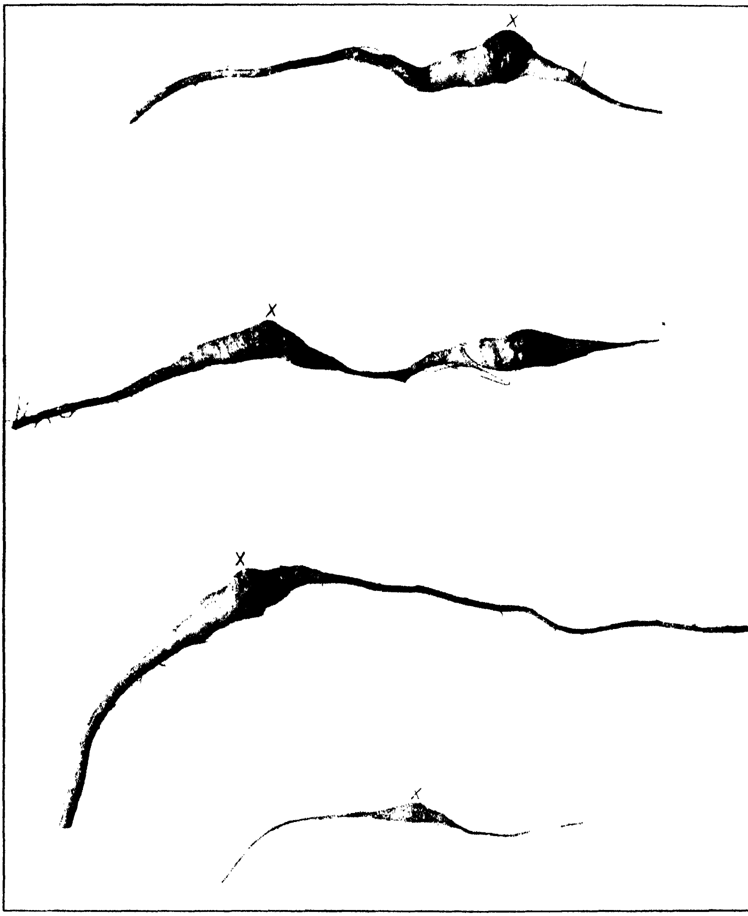
Plasmodiophora brassicae:

Three clubs, each of which has resulted from the original infection of a small bit of tissue on one side of the stem. They are spindle-shaped and are thickest on the side to which the inoculum was sealed.

PLATE 63

Plasmodiophora brassicae:

Some typical spindle-shaped clubs taken from cabbage plants grown in infected soil. The points at which the parasite is believed to have entered the roots are marked by the letter X. Note that the clubs are thickest at these points.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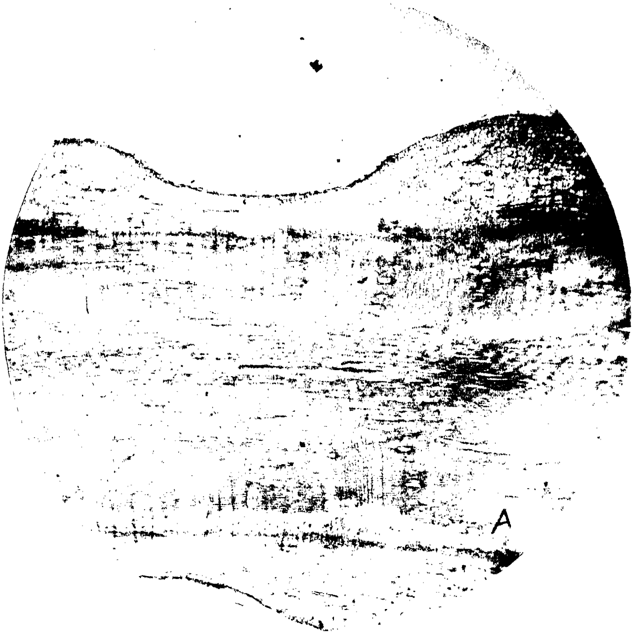
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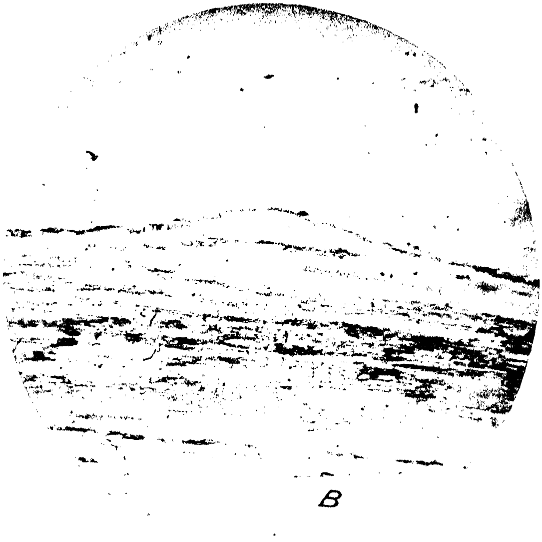
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PLATE 64

Plasmodiophora brassicae.

A.—A longitudinal section through a young cabbage stem. Infection has taken place at two points on the stem and the swellings are about to fuse together. The cambium between the two swellings is already infected. × 12.

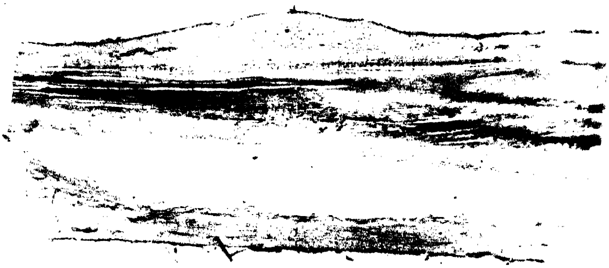
B.—A longitudinal section through a cabbage stem 11 days after inoculation of a single small spot on the stem. The infected tissues are beginning to respond to the parasite. Only the outer portions of the cortex are infected at this time. × 12.

PLATE 65

Plasmodiophora brassicae:

A.—A section through a cabbage stem 13 days after inoculation. × 12.

B.—A section 15 days after inoculation. Note that the disease has spread deeper into the tissues. In figure A the plasmodia are just beginning to infect the cambium in figure B they may be seen in the tissues beneath the cambium. × 12.



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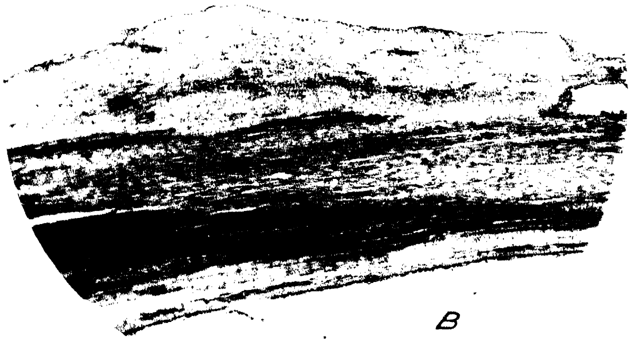


B

A

B

PLATE 66

Plasmodiophora brassicae:

A.—A section through a cabbage stem 17 days after inoculation. The swelling is increasing, and the parasite may be seen spreading along the cambium. × 12.

B.—A section through a stem 19 days after inoculation. The swelling is much larger than it was two days earlier. The plasmodia are also getting larger, as is shown by the size ot the dark bodies in the cells. × 12.

PLATE 67

Plasmodiophora brassicae:

A.—A section through a cabbage stem 21 days after inoculation. The disease has spread around the stem and the plasmodia may be seen in the cambium opposite the large swelling. × 12.

B.—A stage in the infection of the cambium. The plasmodia have not yet begun to pass out into the cortex or in toward the wood. Note that the cambium is infected far beyond the region of swellings. × 18.

A

B


A


B

PLATE 68

Plasmodiophora brassicae:

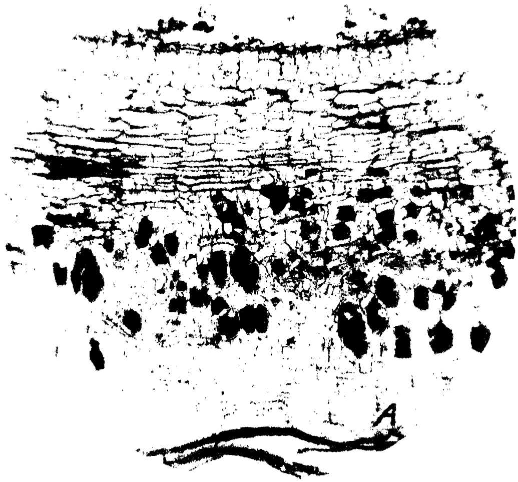
A–B.—Both figures on this plate show cross sections through cabbage stems that became infected when rather old. The disease is confined to those tissues that were undifferentiated at the time of infection or that have developed since infection took place. × 70.

PLATE 69

Plasmodiophora brassicae:

A.—A longitudinal section through a cabbage stem that became infected after the vascular elements were well differentiated. Note that the disease is confined to a definite band of tissue between wood and bark. ×70.

B.—A section through one of the knoblike branch roots that are produced on infected roots. ×60.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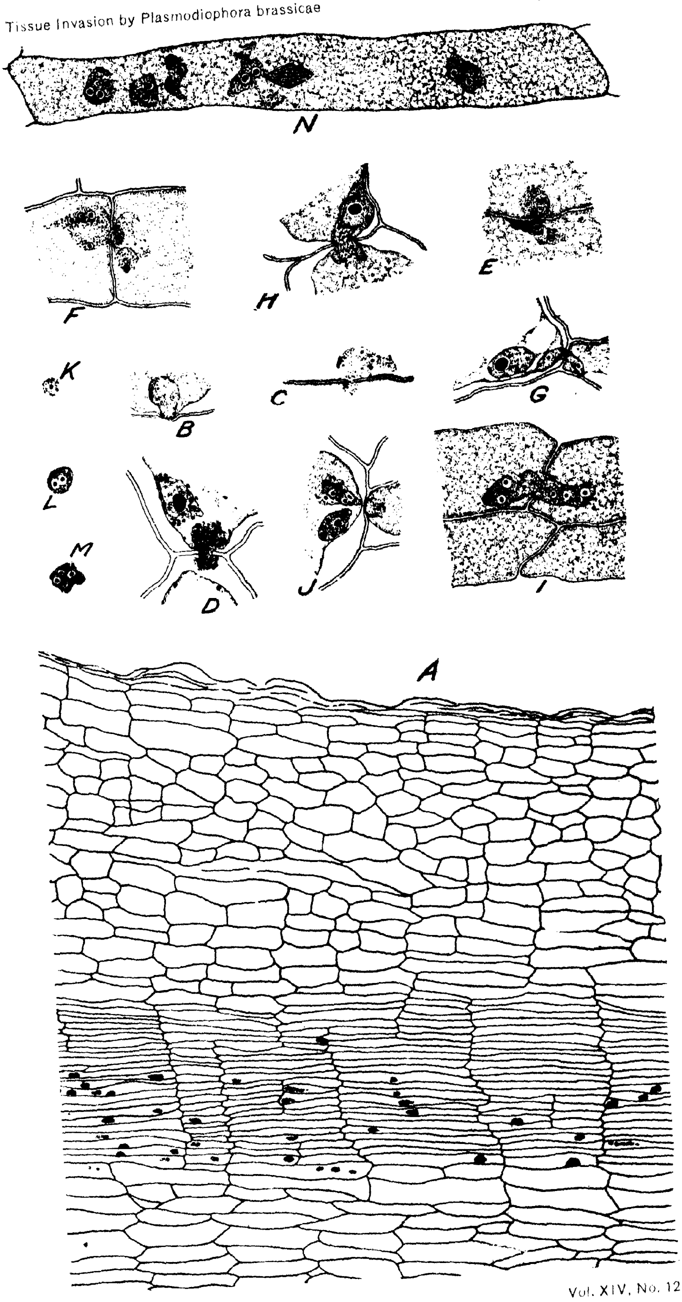
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PLATE 70

Plasmodiophora brassicae:

A.—A portion of a longitudinal section through the stem of a young cabbage plant. The dark bodies represent plasmodia; host-cell contents are not shown. At this stage only the cambial region is infected. ×193.

B.—What is believed to be a very early stage of cell-wall penetration. The plasmodium is closely applied to the cell wall, which seems to be softening and is bending.

C.—An early stage in passage through a cell wall. An opening has been made in the cell wall through which the plasmodium is beginning to pass.

D.—A little later stage than that shown in C. Here the plasmodium has started to pass through the wall, but has not yet penetrated the protoplast of the new host cell.

E, F.—Still later stages in the passage through cell walls.

G.—Interesting because a nucleus is passing through the opening in the wall.

H.—A case in which the opening made in the cell wall is unusually large.

I.—Plasmodium passing through the end of a cell in the region of the cambium.

J.—A case in which plasmolysis of the host cells seems to have broken a migrating plasmodium into two parts.

K.—An ameba taken from a cambium cell.

L, M.—Two small plasmodia that were found in cambium cells far from the point of original penetration.

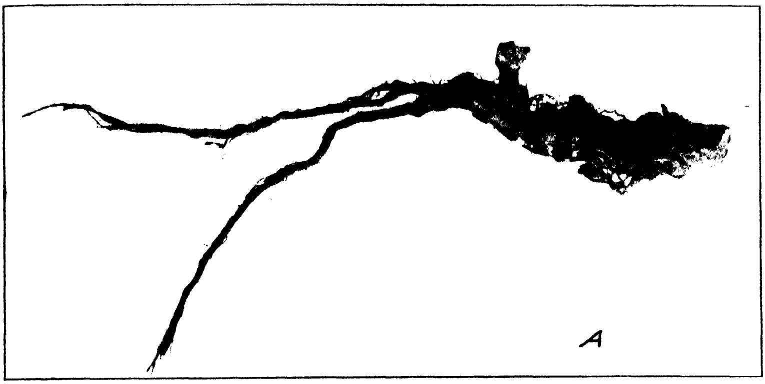
N.—Infected cambium cell. The host nucleus is in process of division. ×836.

PLATE 71

Plasmodiophora brassicae:

A.—A young shoot arising from a diseased lateral root of cabbage.

B.—Two large diseased shoots coming from diseased tissue. Here the diseased leaves are quite large and are green.


A


B

A

B

PLATE 72

Plasmodiophora brassicae:

A.—A section through a portion of an infected green cabbage leaf. × 40.
B.—An infected shoot that is growing downward.

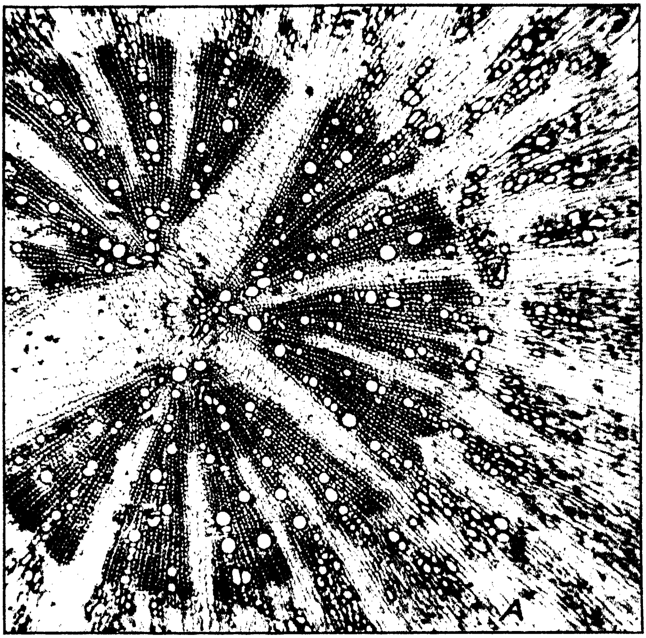
PLATE 73

Plasmodiophora brassicae:

Stages in the infection of medullary rays

A.—A rather large woody cylinder that is beginning to split apart through the abnormal growth of its medullary rays. Several of the rays are being invaded by the parasite. $\times$ 40.

B.—The woody cylinder of a cabbage root. It is being split into two almost equal parts by the growth of medullary tissue. $\times$ 40.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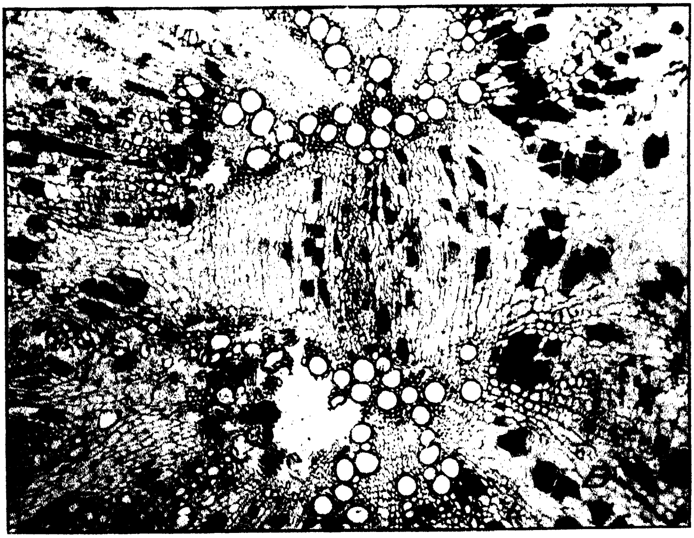

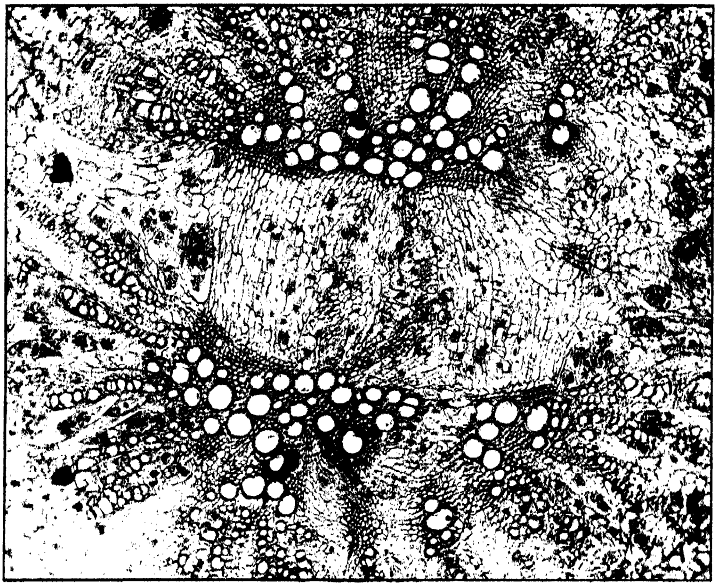

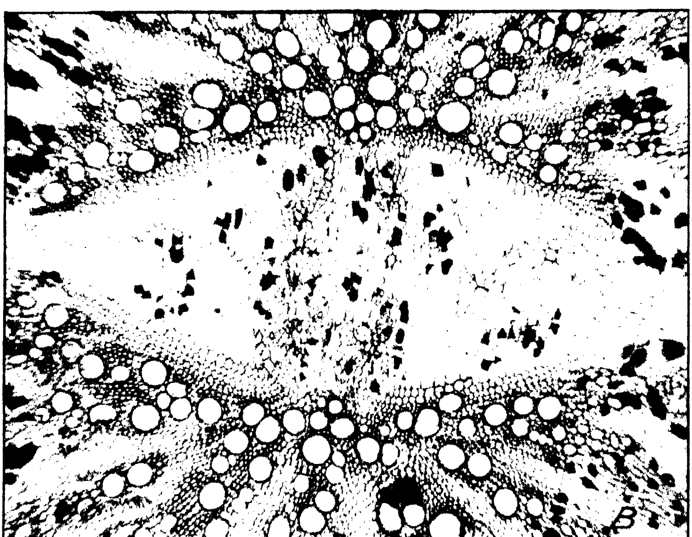
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PLATE 74

Plasmodiophora brassicae:

A.—Somewhat later stages of medullary growth than those shown in Plate 73. The cylinder was first split into two halves. Note that the bundles of the lower half are being still further split up. $\times$ 35.5.

B.—Another cylinder being split into two equal halves. This figure shows how the medullary cells increase in size during the first two or three divisions. Two or three rows of cells bordering on the diseased tissue still have the characteristics of medullary ray cells, but the cells of the row adjoining the diseased tissue are much larger than ray cells. $\times$ 40.

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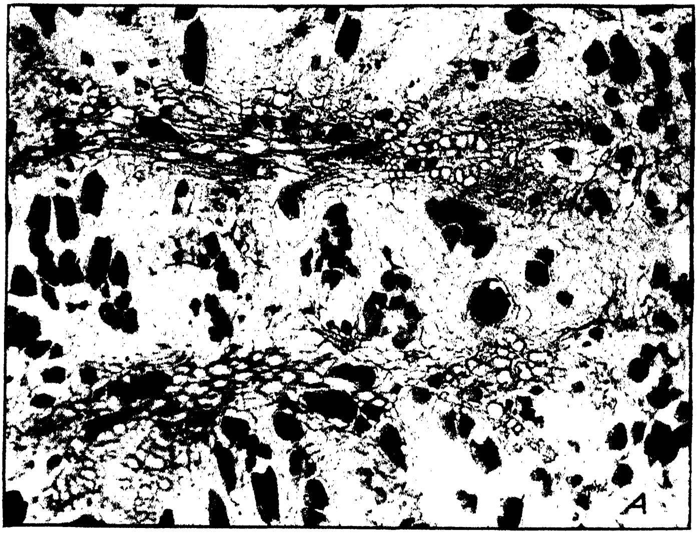
PLATE 75

Plasmodiophora brassicae:

A.—The wood of an old cabbage stem that is being split apart by medullary infection. $\times$ 40.

B.—A somewhat later stage. Here the two woody halves are being forced still farther apart. $\times$ 40.



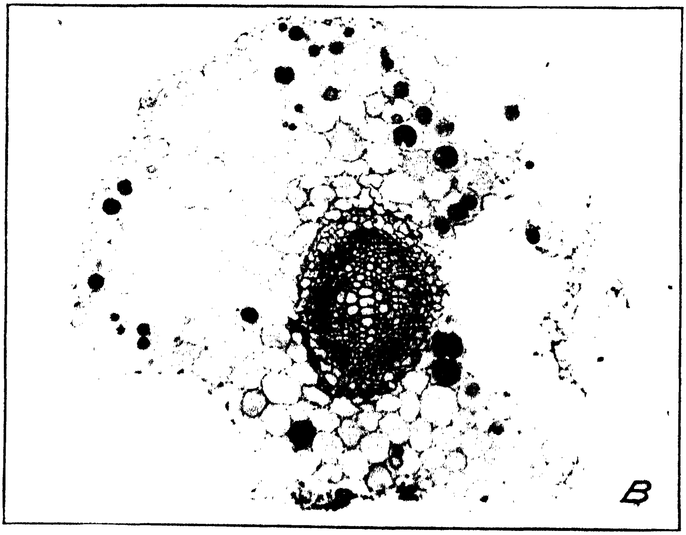


PLATE 76

Plasmodiophora brassicae:

A.—A longitudinal section through the woody part of a cabbage stem that has been split open by medullary infection. × 70.

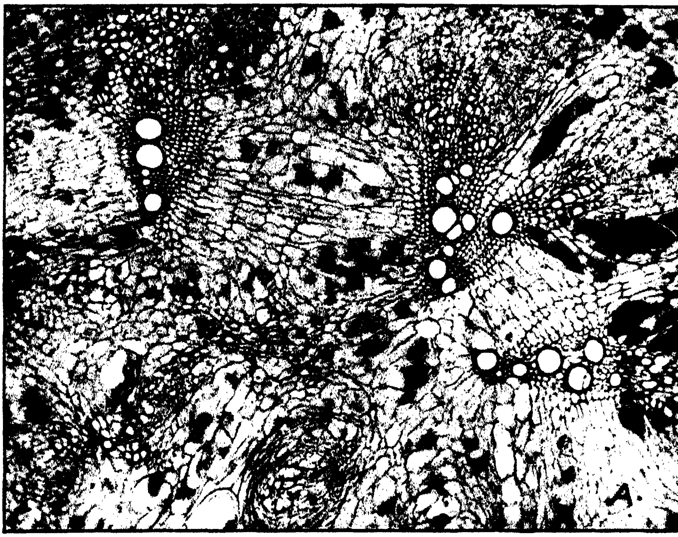
B.—A cross section of a young cabbage root. The ragged outer tissues are the primary cortex. The dark, more or less spherical bodies in the cells of these tissues are the plasmodia of *Olpidium brassicae*. × 80.

PLATE 77

Plasmodiophora brassicae:

A.—Several xylem strands being forced apart by medullary infection. $\times$ 40.

B.—A bundle that is beginning to be fan-shaped in cross section. This is the result of growth and cell differentiation after the bundle had split off from other bundles. $\times$ 40.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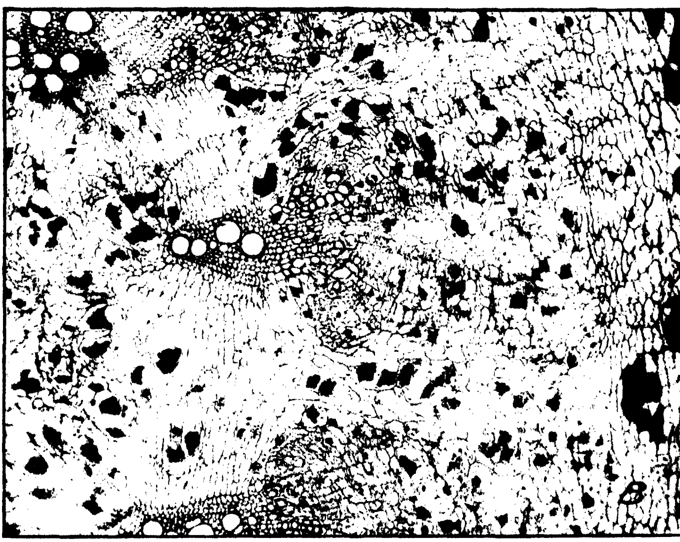
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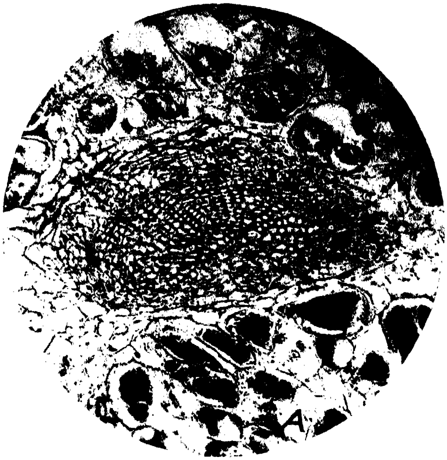
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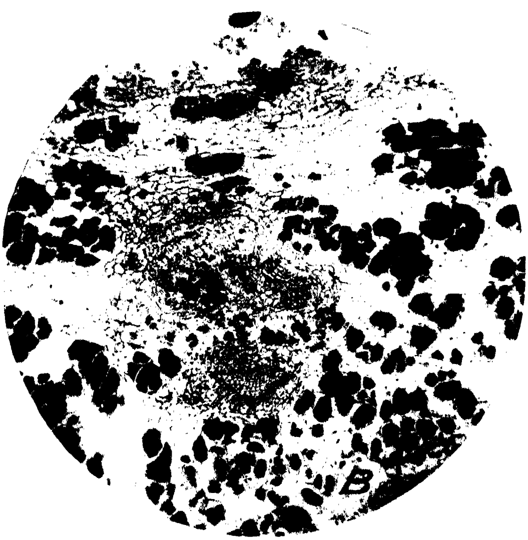
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PLATE 78

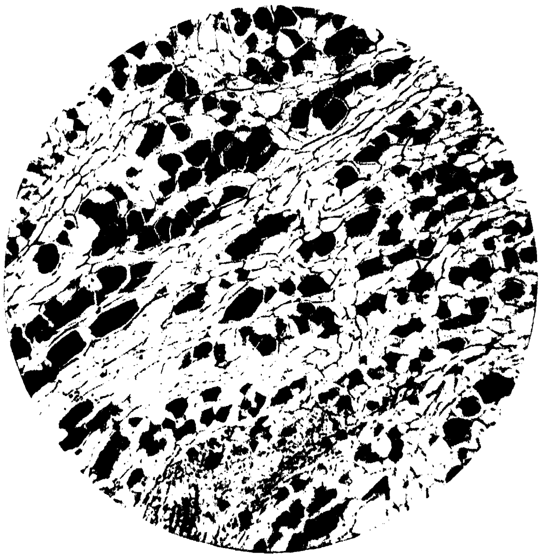
Plasmodiophora brassicae:

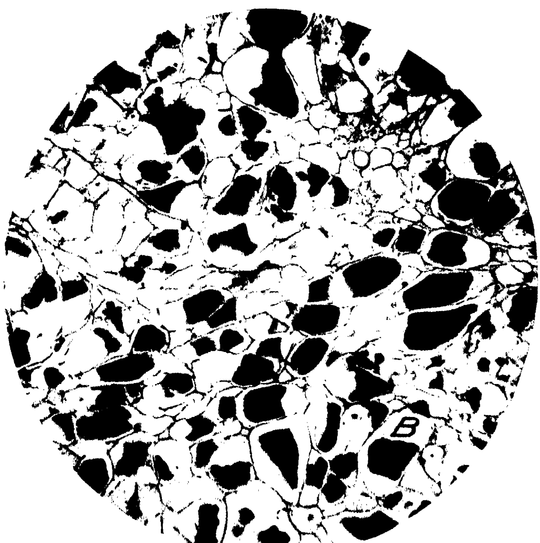
A.—A fibrovascular bundle that is almost semicircular in cross section. × 100.
B.—A strand that is almost circular in cross section. × 50.

PLATE 79

Plasmodiophora brassicae: Distribution of the parasite in the tissues of two different clubs at the time of spore formation—

A.—30.9 per cent of the surface of the photograph is occupied by spore masses.
B.—28.8 per cent of the photograph is occupied by these masses. × 55.

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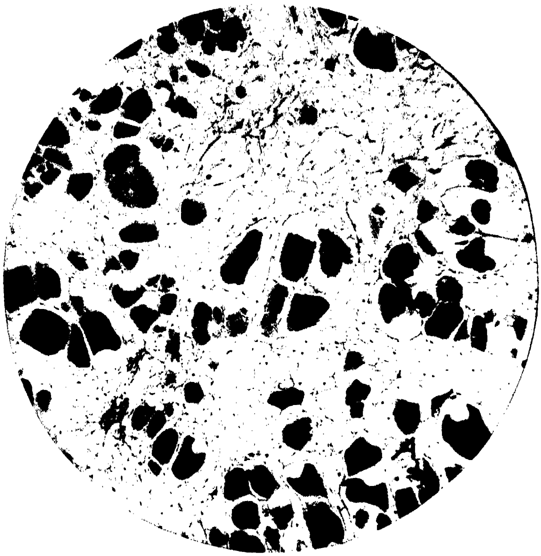

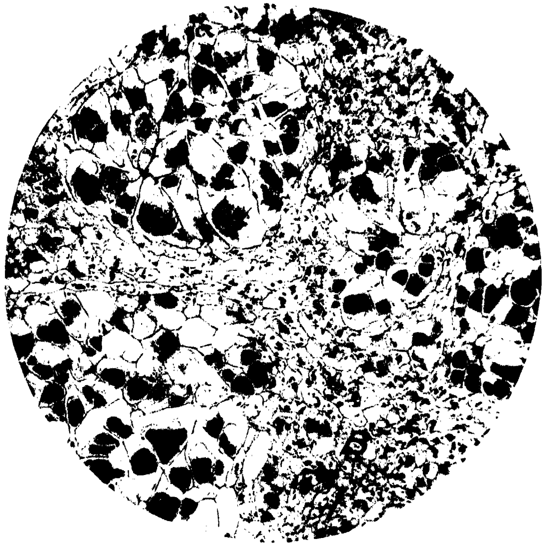

PLATE 80

Plasmodiophora brassicae: Tissues from two other clubs—

A.—28.8 per cent of the photograph is occupied by the spore masses. Most of the cells are free of infection. × 55.

B.—An unusual distribution of the parasite. Here practically all cells are infected. × 55.

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JOURNAL OF AGRICULTURAL RESEARCH

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AN IMPROVED METHOD FOR RECOVERING TRYPANOSOMES FROM THE BLOOD OF RATS FOR ANTIGEN PURPOSES IN CONNECTION WITH COMPLEMENT FIXATION

By F. H. REYNOLDS and H. W. SCHOENING, *Pathological Division, Bureau of Animal Industry, United States Department of Agriculture*

UNDESIRABLE FEATURES OF METHODS IN USE

Up to the present time the methods employed in recovering trypanosomes from the blood of artifically infected rats, though quite satisfactory, have presented some features which were somewhat detrimental to the antigen and which, if overcome, would greatly improve the same as to purity and, obviously, specificity. The question of suitable and sufficient antigens being one of vital importance, more especially in this laboratory, where many thousands of fixation tests for dourine are performed annually, it was desirable to seek an improvement.

Watson [1] describes a method whereby the trypanosomes might be recovered in large quantities from the blood of infected rats killed at the height of the disease. It was essentially a method of repeated centrifuging, as the erythrocytes, being of greater specific gravity than the trypanosomes, necessarily settled to the bottom. However, the method had its faults, in that many of the organisms would be drawn down with the red cells and it was necessary to sacrifice many of the trypanosomes to prevent the presence of too great a quantity of red cells in the antigen. Therefore it was necessary to eliminate the following undesirable features: First, however careful and painstaking one might be, it was quite impossible to collect all the trypanosomes present in the blood; second, it was impossible to procure a pure antigen totally devoid of rat erythrocytes which, when present in large numbers, would tend to decrease the antigenic value as well as the keeping qualities; third, it was a laborious process consuming half a day, and, as experience has shown that subjection to room temperature greatly impairs the antigenic value, the necessity for its rapid preparation and early storage on ice is quite apparent. Even then it was quite impossible to procure a pure product. There-

[1] WATSON, E. A. DOURINE AND THE COMPLEMENT FIXATION TEST. *In* Parasitology, v. 8, no. 2, p. 156–183. 1915.

fore, with a view to overcoming the several obstacles attending the preparation of the trypanosomal antigen, the following technic has been devised and has given good results.

TECHNIC OF NEW METHOD

Blood of infected rats is collected in a 1 per cent sodium-citrate solution in physiological salt solution in order to prevent coagulation. When all the blood has been collected, the solution is filtered through cheesecloth to remove clots, fibrin, etc., poured into tubes, and centrifugalized for about 20 minutes at 2,100 revolutions per minute. This precipitates all the corpuscles and most of the trypanosomes, leaving an upper stratum of blood serum and citrate solution containing some of the organisms. This fluid is drawn off and again centrifugalized in order to recover any of the protozoa which may be present. To the other tubes containing the mass of corpuscles intermixed with and superimposed by trypanosomes is added sufficient distilled water to produce complete hemolysis of the rat erythrocytes, a matter of about 20 minutes, which procedure is facilitated by agitation of the mixture in a flask. This also is centrifugalized but, in this instance for about half an hour, upon the completion of which there is found at the bottom of the tubes a mass of trypanosomes with an admixture of stroma of the hemolyzed red cells, which latter, in quantity, has been found to be negligible. After discarding the supernatant fluid (hemoglobin-stained water) physiological salt solution is added and the material vigorously shaken until the mass of trypanosomes is disintegrated and evenly distributed throughout the solution. Centrifuging is again resorted to with similar results, the washed mass of trypanosomes being packed at the bottom of the tubes. The salt solution is poured off and an amount of preserving fluid (physiological salt solution and glycerin aa) equal to about twice the amount of trypanosomes added; the mixture is then agitated until a uniform suspension is acquired, when it is stored at a low temperature until used.

In order to determine whether the use of distilled water in laking the corpuscles would have any detrimental effect on the trypanosomes as regards their antigenic value, immediately following their preparation, and also after an interval of two weeks, the following procedure was employed:

Twenty white rats were inoculated subcutaneously with a suspension of *Trypanosoma equiperdum* in salt solution. At the end of the third day the blood of all the animals showed a heavy infestation with the protozoa.

The animals were then bled to death into 300 cc. of a 1 per cent sodium-citrate solution in physiological salt solution, which was then divided equally and placed into two flasks.

The trypanosomes in flask 1 were recovered by repeated centrifugalization, the machine being run a sufficient length of time to drive all the corpuscles to the bottom of the tube, but leaving a large number of trypanosomes in the supernatant fluid. This was pipetted off and the blood mixed with salt solution and again centrifugalized. By repeating this operation a number of times, as many trypanosomes as possible were obtained in suspension. This fluid was then centrifugalized, driving all the trypanosomes to the bottom of the tube. The supernatant fluid was discarded and the trypanosomes were suspended in the following solution: Glycerin 5 cc., physiological salt solution 5 cc., and labeled "Antigen I."

The trypanosomes in the second flask were recovered by breaking up the red blood cells with distilled water in the manner hereinbefore described. These trypanosomes were also suspended in 5 cc. of glycerin and 5 cc. of physiological salt solution and labeled "Antigen II."

On inspection of the two antigens it was readily seen that Antigen II contained many more trypanosomes than Antigen I and apparently no blood corpuscles, while Antigen I showed the presence of considerable blood which it had been impossible to get rid of without sacrificing many of the trypanosomes.

Both antigens were titrated for antigenic strength and anticomplementary action immediately after preparation and after being stored in the ice box for two weeks. Tests for hemolytic action were also made.

The hemolytic system employed consisted of a 3 per cent suspension of sheep red cells, 2½ units of hemolytic amboceptor, and 1½ units of complement, the latter being titrated against the amboceptor and sheep cells.

Both antigens were diluted 1 to 10 with physiological salt solution.

The test for antigenic power was made against 0.15 cc. of known positive dourine serum, which was the pooled serum from 20 horses affected with dourine.

In the test immediately after preparation the antigenic unit of Antigen I was 0.25 cc. and the anticomplementary unit was 2 cc., and was not hemolytic in five times this amount. The antigenic unit of Antigen II was 0.05 cc., the anticomplementary unit 3 cc., and was not hemolytic in five times this amount.

Two weeks after preparation the antigenic unit of Antigen I was 0.35 cc., the anticomplementary unit 1.5 cc., and the antigen showed no hemolytic action. The antigenic unit of Antigen II was 0.1 cc., the anticomplementary unit 3 cc., and it showed no hemolytic action.

The results are compared in the following table:

Antigen.	Antigenic unit.	Anticomplementary unit.	Hemolytic action.
Immediately after preparation:	*Cc.*	*Cc.*	
Antigen I	0.25	2.0	None.
Antigen II	.05	3.0	None.
Two weeks after preparation:			
Antigen I	.35	1.5	None.
Antigen II	.10	3.0	None.

The use of distilled water in laking the red blood cells had no detrimental effect on the trypanosomes with regard to their antigenic value.

CONCLUSIONS

In concluding, the following advantages of the new method are pointed out:

(1) The antigen is freed of all erythrocytes.

(2) All the trypanosomes present in the blood are recovered.

(3) The keeping quality is improved.

(4) The time consumed is about one and one-half hours, with practically no effort, as compared with four or five hours.

(5) The antigenic power is increased and the anticomplementary action diminished.

LIFE HISTORY OF PEMPHIGUS POPULI-TRANSVERSUS

By THOMAS H. JONES[1]

Entomological Assistant, Truck-Crop Insect Investigations, Bureau of Entomology, United States Department of Agriculture

WITH TECHNICAL DESCRIPTIONS BY C. P. GILLETTE, *Collaborator, State Entomologist of Colorado*

INTRODUCTION

The first collection in Louisiana of aphids belonging to the genus Pemphigus from the roots of plants of the family Cruciferae seems to have been made on November 19, 1914, by Mr. E. S. Tucker, formerly Associate Entomologist of the Louisiana Experiment Stations. These specimens were taken from cabbage roots (*Brassica oleracea capitata*) at Loranger, in Tangipahoa Parish. Shortly afterwards root aphids of the same genus were noted at Baton Rouge by the writer, and material for identification was submitted to specialists acquainted with the genus. As the stages sent were not recognized as belonging to any described species, and as there were apparently no published records of any species of Pemphigus occurring on crucifers in the United States, studies concerning the life history, habits, and economic importance of the species were begun.

After the writer had begun the investigation Maxson (*23, p. 501*)[2] published an article on *Pemphigus betae* Doane in which he stated that—

> Lice of this genus have been repeatedly taken on turnips in the south.

In a letter to the writer Mr. Maxson has given the additional information that—

> the specific instances referred to were brought to my attention by Mr. F. B. Paddock, State Entomologist of Texas, he having sent material to me for identification.

[1] The writer wishes not only to thank Dr. Gillette for his kindness in drawing up the descriptions and supervising the preparation of illustrations, but also for suggestions regarding the biological studies, and especially for the interest which he has taken in the work. Mr. J. J. Davis, of the Bureau of Entomology, and Mr. J. R. Parker, of the Montana Agricultural Experiment Station, also have shown much interest in these studies, and the writer desires to thank them for the aid they have given. Messrs. C. E. Smith and J. L. E. Lauderdale, while members of the Bureau of Entomology, rendered valuable assistance in the studies of the life history and habits of the species.

[2] Reference is made by number (italic) to "Literature cited," p. 592-593.

Dr. C. P. Gillette also informed the writer that Mr. Paddock sent him material from Texas collected from turnip roots on February 13, 1914, and that the species is the same as that which attacks crucifers in Louisiana. Mr. L. C. Bragg has collected the species from watercress (*Roripa* sp.) near Fort Collins, Colo. Mr. J. J. Davis, of the Bureau of Entomology, has written that several years ago he received a species of Pemphigus collected on curly turnip (*Brassica rapa*) at Greenwood, Miss., which seems to agree with the one under discussion.

It is the purpose of this paper to present the results of investigations carried on at Baton Rouge regarding the life history and habits of the species, together with records from other points in Louisiana and from other States. These results indicate that the species of Pemphigus that feeds on the roots of crucifers is identical with the well-known *Pemphigus populi-transversus* Riley, which causes galls on the leaf petioles of some of the poplars or cottonwoods (*Populus* spp.). Fortunately, it has been possible to have these notes accompany descriptions of the various stages made by Dr. Gillette.

HISTORICAL REVIEW

While *Pemphigus populi-transversus* has been often referred to by entomological writers since Riley described the species in 1879 (*1, p. 15–16; pl. 2, fig. 5, a–c*), these references have been brief and for the most part have been limited to records of the occurrence of the species in some State or particular locality and a few words regarding bibliography and the previously recorded hosts and distribution. Such references are mentioned under "Distribution and hosts" on page 580.

In works in which insects are treated especially from an economic standpoint there are several references to the species and its gall. In 1890 Packard (*3, p. 434*) mentioned it in his work, "Insects injurious to forest and shade trees." Bruner (*6, p. 218, fig. 57*), included it in an article entitled "Insect enemies of ornamental and shade trees growing in cities and parks," which was published in 1893 in the Annual Report of the Nebraska Horticultural Society, and again mentioned it (*11*) in an article on aphids which appeared in The Nebraska Farmer in 1901. Lintner (*9, p. 361–362*) included it in his Thirteenth Report of the State Entomologist of New York, published in 1898, and stated that it had been abundant on *Populus monilifera* in Washington Park, Albany, N. Y., during 1896 and 1897. Felt (*14, p. 247, 620, 635–636, pl. 11, fig. 15, 16*) in his work, "Insects affecting park and woodland trees," published by the New York State Museum in 1906, also referred to its abundance in the vicinity of Albany and gave colored figures of the gall. Baldwin (*21, p. 208*) referred to it in the Fifth Annual Report of the State Entomologist of Indiana in 1912 and gave two original illustrations of the gall.

In lists and synopses of North American aphids the species has been mentioned by several authors. It is included in the "Host-plant list of North American Aphididae" by Williams (*4, p. 6, 9*), published in 1891, and in a bulletin entitled "The Aphididae of North America" by Hunter (*10, p. 78*), issued in 1901. In a synopsis of the genus Pemphigus Jackson (*14, p. 182, 183, 206–208*) in 1908 referred to the species somewhat in detail and stated that the life history was "very imperfectly known." Further references to its life history have been made by Davidson (*16, p. 372*) and Gillette and Bragg (*22, p. 98*). Davidson stated that the stem mothers had been observed "founding their colonies" in the vicinity of Stanford University, California, in March. Gillette and Bragg (*22, p. 98*) in "Notes on some Colorado aphids having alternate food plants," published in 1916, gave "winter host, Populus species; alternate host unknown."

EXPERIMENTS IN TRANSFERRING THE SPECIES FROM CRUCIFERS TO POPLAR AND FROM POPLAR TO CRUCIFERS

Soon after investigational work on this root aphid was begun, winged viviparous females (winged migrants or sexupara), collected from the soil about cruciferous roots, were sent to Dr. Gillette and to Mr. J. R. Parker. Both stated that they did not recognize the individuals as belonging to any described species of the genus Pemphigus, but suggested that it was possible that they might be identical with *Pemphigus populi-transversus*. In view of this, experiments were begun in an attempt to ascertain whether there was a migration of the species from crucifers to poplar at one season of the year, and a return migration at another season.

In 1916 cuttings were taken from trees of *Populus deltoides*, before the buds began to swell, and stuck in moist sand in flowerpots, which were kept in a greenhouse. When leaves began to appear on these cuttings, young individuals of the stem mother (fundatrix) were placed on them. These stem mothers had recently issued from eggs obtained in the laboratory from the true sexes, which had in turn been produced by winged females (sexupara) taken from about the roots of crucifers in the field. Swellings soon began to appear on the petioles where the stem mothers had located, and these swellings, increasing in size, gradually took on an appearance typical of the gall of *Pemphigus populi-transversus* (Pl. 82). Unfortunately the stem mothers died after the galls had reached a diameter of about 3/8 inch. Galls were again formed about stem mothers on leaf petioles of poplar in the greenhouse in the spring of 1917, and these, developing to a greater size, appeared identical to those of *P. populi-transversus*.

During the fall of 1916 winged migrants (fundatrigenia) from the galls of *Pemphigus populi-transversus* were placed under cheesecloth in a cage where turnips were growing. Later, examination of the soil showed the roots of the turnips to be heavily infested with aphids of the genus

Pemphigus, the infestation being similar to that which occurs on cruciferous roots under field conditions. It was evident, when the growth of the plants in this box was compared with that made by plants of the same age in an uninfested box, that they had been affected by the presence of the aphids at their roots (Pl. 84, F). In fact, the turnips died before winged migrants appeared, though probably not altogether because of the insect infestation.

Winged migrants from galls were also placed on turnips growing in pots in the greenhouse. The soil in these pots had been heated previously to a temperature sufficient to kill insect life, and the pots were covered with cloth-covered wire frames. Later, examination showed wingless individuals of *Pemphigus* sp. to be present on the roots, but again the plants died before the winged forms appeared. Before the plants died, however, a number of the wingless aphids were transferred to pots covered with cloth containing cabbage plants, the roots of which had been dipped in a mixture of water and nicotine sulphate before they were planted. On March 2 of the following year winged viviparous females, such as are found in the colonies at the roots of crucifers, were observed. No aphids appeared on the roots of control plants.

These experiments, together with others that have been carried on, indicate that the forms found on poplar and on crucifers belong to the same species. Additional proof is furnished by the fact that from poplar trees in the spring were taken winged viviparous females which agree with winged migrants found at cruciferous roots at that time of the year, and that during late summer and during the fall there were collected from the leaves of crucifers winged viviparous females which are identical with those found in galls of *P. populi-transversus*.

DISTRIBUTION AND HOSTS

The species has been recorded as occurring on poplar in California (*16, p. 372; 19, p. 398; 20, p. 699*), Texas (*1, p. 15–16*), Colorado (*1; 7, p. 116; 22, p. 98*), Kansas (*12, p. 22, 23*), Nebraska (*18, p. 12*), Missouri (*1, p. 15–16*), Iowa (*5, p. 130*), Minnesota (*2, p. 20, 21*), Illinois (*17, p. 411*), Indiana (*21, p. 208*), New York (*9, p. 361–362; 13, p. 247, 620, 635–636; 15, p. 355*), and Massachusetts (*15, p. 355*). Mr. Parker has collected it at Lovell, Wyoming, Dr. Gillette has specimens from Arizona, and Mr. Davis writes that he has records of its occurrence in Wisconsin, Michigan, and Ohio. Mr. H. F. Wilson has taken it in Wisconsin, and the writer has seen galls, apparently made by this species, at Agricultural College, Miss., and Jacksonvllle, Fla.

Four species of the genus Populus, *balsamifera*, *monilifera*, *trichocarpa*, and *fremontii*, have been mentioned as hosts. Britton and Brown (*8, p. 491, fig. 1165; p. 493, fig. 1172*) give the following distribution for *P. balsamifera:*

Newfoundland to Hudson Bay and Alaska, south to Maine, New York, Michigan, Idaho, and British Columbia.

They give *P. monilifera* as a synonym under *P. deltoides* (the name used in this article), a species which, they state, occurs from Quebec to the Northwest Territory, south to New Jersey, Florida, Colorado, and New Mexico. *P. trichocarpa* and *P. fremontii* apparently occur in the Western States.

FORMATION OF GALLS

In transferring the species from crucifers to sprouting cuttings of *Populus deltoides* it was found that the petioles of young leaves, just out of the bud, are apparently the only ones upon which galls begin to develop. A transverse groove first appears on the petiole where the young stem mother has located, the developing petiole gradually bending at this point. The tissue surrounding the groove, which is on the inside surface of the bent petiole, gradually enlarges until a hollow globular gall, with a transverse slit on the surface opposite the petiole, is formed around the stem mother. Galls found in the field on May 17 were roughly spherical in shape and varied in diameter from ¼ to ½ inch. Just before the leaves fall from the trees some of the galls reach a greatest diameter of nearly an inch. The galls vary considerably in shape, length of the transverse slit, and development of the lips (see Pl. 83; 84, A–E). While the general color of the gall is the same as that of the petiole, a portion of the surface often has a reddish tinge.

Practically all of the galls occur somewhere on the leaf petioles, though what seem to be the galls of this species have been found on the new stem growth to which the petioles are attached. The galls usually occur singly, but as many as three have been seen in juxtaposition on one petiole.

In midsummer the gall contains, and indeed is often filled with, the comparatively large stem mother, her progeny in various stages of development, all covered with waxy secretion, together with molted skins and usually with liquid globules.

DATES WHEN GALLS ARE FOUND AT BATON ROUGE

In 1917, at Baton Rouge, winged viviparous females and the true sexes produced by them were taken in a small cavity on the trunk of a poplar tree as early as March 8. These winged females are identical with the winged migrants (sexupara) found about the roots of crucifers. On March 28, young stem mothers were found on leaf petioles where, to judge from the size of the galls, they had apparently been present for a few days only.

During 1916, galls were noted on May 17 which had reached a diameter of ¼ to ½ inch. On November 7, when a large percentage of the leaves of the poplar had fallen, few galls remained on the trees.

PERCENTAGE OF LEAF PETIOLES SHOWING GALLS

Leaves were gathered from a tree of *Populus deltoides* on July 26, 1916, to ascertain the percentage of leaf petioles having galls upon them, and to learn whether galls occurred in greatest numbers in any one portion of the tree as regards the height above the ground. Galls are found upon comparatively small trees and upon the largest specimens.[1] The tree selected was about 30 feet in height and the infestation appeared to be an average one for the particular locality. Branches were broken from it at random at various heights. The leaves were then stripped from the branches and counts made of those leaves showing galls and those not infested. It was found that there was a considerable variation in the percentage of leaves showing galls on different branches, but the distance above the ground apparently had no bearing upon the percentage of leaves infested. The results are given in Table I.

TABLE I.—*Percentage of leaf petioles of Populus deltoides infested with Pemphigus populi-transversus, Baton Rouge, La., July 26, 1916*

Position of branch on tree.	Number of branches examined.	Number of leaves examined.	Number of leaf petioles showing galls.	Percentage of leaf petioles showing galls.
In approximate lower third	5	222	54	24.3
In approximate middle third	5	441	117	26.5
In approximate upper third	5	512	137	26.7
Total	15	1,175	308	25.2

DATES WHEN WINGED MIGRANTS (FUNDATRIGENIA) ARE FOUND IN GALLS

At Baton Rouge winged females begin to appear in the galls on *Populus deltoides* at a somewhat earlier date than that recorded for other localities. Riley (*1, p. 15–16*), in connection with his original description of the species, in which he gives Missouri, southern Texas, and Colorado as localities where this species of Pemphigus occurs, states that the winged females are "produced in autumn, sometimes not until the leaves have fallen." Williams (*18, p. 12*) mentions finding winged females, "evidently but lately transformed," in galls at Ashland, Nebr., on September 25. At Baton Rouge, in 1916, winged females were found in the galls as early as June 1, though a very small percentage of the galls contained such individuals on this date. Not until September were they present in more than 10 per cent of rather large collections examined.

[1] Three other species of aphids belonging to the subfamily Pemphiginae have been collected from *Populus deltoides* at Baton Rouge. Of these, *Pemphigus populicaulis* Fitch is the most common, although it is much less abundant than is *P. populi-transversus*. The two other species, found only occasionally in galls which they form on the leaves, have not been identified.

Of 200 galls collected on September 15, 17 per cent contained winged females, while they were present in all of 150 galls taken on September 28.

The number of winged females in a single gall also showed a gradual increase as the year advanced. In June usually only one was found in a gall, while on November 7 as many as 76 were present.

DATES WHEN WINGED MIGRANTS (FUNDATRIGENIA) LEAVE GALLS

Although winged females appeared in the galls at Baton Rouge during 1916 as early as June 1, there is no evidence to show that migration to crucifers took place until late summer. The earliest collection of winged female migrants on crucifer leaves was on August 31, and not until early October were crucifer roots found infested to any considerable extent. On October 2, 1917, during a period of clear, cool, autumn weather, the migrants from the galls were common on turnip leaves at least 500 feet from poplar trees. As many as five were found on the underside of a large leaf. The greatest migration probably occurs during October. While the writer has no definite data regarding the maximum distance they may traverse while in flight, it is probable that they, as well as the sexupara, may be carried long distances by winds.

NUMBER OF WINGLESS VIVIPAROUS FEMALES (VIRGOGENIA) TO WHICH WINGED MIGRANTS (FUNDATRIGENIA) GIVE BIRTH

On October 25, 1916, 25 winged females taken from galls were placed, without food, in vials and kept under observation indoors. All of these began almost immediately to give birth to young, and by October 30 all had died. The average number obtained from each individual was 26, the number ranging from 14 to 37. In one instance a female brought forth 30 young in about 24 hours.

The young viviparous females locate on the roots, feed, and when mature bring forth other wingless viviparous females. In this way the subterranean colonies become established.

INJURY TO AND APPEARANCE OF PLANTS INFESTED WITH SUBTERRANEAN FORMS

While severe Pemphigus infestation on the roots of crucifers may be indicated by the wilted condition of the leaves, a slight or moderate infestation does not usually affect, to a noticeable degree, the portions of the plant above the ground. For this reason such infestation usually goes unnoticed. In other words, this insect, while it may cause as much damage as many of those species which feed upon the leaves or other parts of the plant above the surface of the soil, does not attract as much attention as such species because it works out of the sight of the ordinary observer. Inasmuch as these aphids feed upon the roots, it is to be presumed, however, that any infestation is detrimental to the plant.

Upon examination of the soil about plants attacked by the subterranean forms colonies may be found upon any portion of the root system, but the small rootlets appear to be preferred. Where dead leaves, or other trash, occur on the surface of the soil, there is often a growth of rootlets immediately beneath, and colonies are often found in such locations.

Mr. J. L. E. Lauderdale made an interesting observation at Baton Rouge on March 19, 1917, while examining roots of *Coronopus didymus* in a field of stock beets (*Beta vulgaris*). The beets were growing on ridges where the soil was less moist and less compact than that midway between the rows. Of 25 plants of *C. didymus* growing on the ridges, 24 were infested with *Pemphigus populi-transversus*, whereas only 6 of 25 plants growing in the low ground between the rows had aphids present on their roots. This would indicate that either the compactness of the soil between the rows, or its higher moisture content, or both, was disadvantageous to the development of the aphids.

The white, flocculent material which the aphids secrete is of material aid in locating them. This secretion often occurs in considerable abundance about the colonies (Pl. 85). The light-yellow color, characteristic of the bodies of the wingless females, except in their early stages, usually makes it easy to locate them when the soil about the roots on which they are feeding is carefully examined.

INJURY CAUSED BY THE SUBTERRANEAN FORMS

Where the aphids occur in small or moderate numbers at the roots of plants, it is difficult to estimate the amount of damage done by them. The following extracts from correspondence received by the Louisiana Experiment Stations give information as to injury to crucifers by *Pemphigus* spp. in Louisiana. As only wingless forms were forwarded by the correspondents, it can not be stated positively that the root aphid causing the injury was *P. populi-transversus*, although that is probable.

On November 13, 1915, a correspondent living at Rhoda in St. Mary Parish wrote:

> I am sending you under separate cover a cabbage plant that is badly infected with a small yellow louse, and would ask if you can recommend anything that can be done. The bug or louse is found at the root of the plant, and seems to suck the sap or eat off the roots, as the plant is badly wilted during the warm part of the day, but revives a little at night, until it finally is killed.

On November 22 of the same year a letter was received from New Iberia, Iberia Parish, in which the writer stated:

> Enclosed please find stalk of cabbage with insect at the root that is destroying all my plants.

A report has also been received from Dr. C. E. Mauldin, in charge of the Iberia Live-Stock Experiment Farm of the Bureau of Animal Industry, at Jeanerette, La., that—

It has been necessary for us to abandon the planting of rape and kale at this station on account of the root-louse.

At Baton Rouge the subterranean forms apparently cause more severe injury to turnip than to any other cultivated cruciferous crop that has been under observation. Not only have they been found in greatest numbers on turnips but plants have been frequently noted which, when pulled, came up easily. The roots of these plants were mostly dead, apparently because of the attack of the aphids.

FOOD PLANTS OF THE SUBTERRANEAN FORMS

Wingless specimens of the genus Pemphigus have been taken in Louisiana from the roots of the following Cruciferae: Cabbage, turnip, mustard (*Brassica nigra*), cauliflower, and broccoli (*Brassica oleracea botrytis*), Brussels sprouts (*Brassica oleracea gemmifera*), rape (*Brassica napus*), *Coronopus didymus*, *Lepidium virginicum*, and *Roripa* sp. The last three host plants are weeds, the first being common in uncultivated fields at Baton Rouge during the winter months, when the plants are sometimes gathered and used as "greens."

Winged migrants (fundatrigenia) of the species of Pemphigus under consideration have been found at the roots of cabbage, turnip, Brussels sprouts, rape, *Coronopus didymus*, and *Roripa* sp. It is quite possible that further observations will disclose the fact that the species occurs also at the roots of plants not belonging to the family Cruciferae. Mr. Lauderdale has found individuals on the roots of stock beet. Adjoining infested roots of *Coronopus didymus* apparently explained their presence on the beet roots, as examination of the roots of many beets, near which no crucifers were growing, failed to disclose additional instances of infestation.

SPREAD OF SUBTERRANEAN FORMS

Observations made in the field and under laboratory conditions indicate that at least the smaller wingless viviparous females (virgogenia) that are present in the soil during the winter months are capable of considerable locomotion, and that when conditions become unsatisfactory, these individuals seek more suitable locations. During December they have been found in great abundance crawling over the surface of the soil and upon the plants in a field of Brussels sprouts. While carrying on some experiments in the greenhouse about a year later, individuals were found to have left flowerpots in which they were feeding on turnip roots, apparently because the turnips had begun to die as the result of being severely infested with the aphid *Myzus persicae* Sulzer. They were

especially numerous on the highest points of the cloth covering these pots, about a foot above the surface of the soil, and could be easily dislodged by slight puffs of air. Some were found under conditions which indicated that they were about 3 feet away from the nearest point where they could have originated.

As the season advances, the wingless viviparous females give birth to individuals which develop into winged viviparous females (sexupara), which later leave the soil and fly away.

DATES WHEN WINGED MIGRANTS (SEXUPARA) ARE FOUND IN THE SOIL

A few winged females have been found in the soil as early as December 12, and, as is the case with the winged females occurring in the galls, their number gradually increases as the season advances. During 1917, roots of *Coronopus didymus* were examined in the field from time to time with the idea of ascertaining how late in the spring the species occurs in the soil. The last winged individuals were taken on April 9. On April 16 no subterranean forms could be found, though winged migrants were alive on poplar as late as April 30.

From the field observations it appears that these winged migrants fly from the crucifers to the poplar trees during the spring, where, usually in some suitable crevice, they give birth to the true sexes. Winged females, agreeing with the winged females found about cruciferous roots, were found in such locations on poplar trees during March and April, 1917. Some observed on March 20 were dead, with true sexes and eggs located near by.

NUMBER OF YOUNG TO WHICH WINGED MIGRANTS (SEXUPARA) FROM CRUCIFERS GIVE BIRTH

In the laboratory, under conditions quite different from those under which the winged migrants would live in the field, the greatest number of sexed individuals to which a single aphid was observed to give birth was six. Usually this winged form brings forth all of her offspring within a short time and then dies. Those kept in the laboratory were examined daily, all of the young usually being produced from the time of one examination to the next. Individuals of both sexes have come from one winged migrant.

Examination of the abdomens of several winged migrants, collected from soil about the roots of crucifers, showed them to contain from four to seven sexed specimens, seven being the predominating number.

DEVELOPMENT OF THE TRUE SEXES (SEXUALES)

Eggs have been obtained in the laboratory from true sexes kept without food. The number of molts which the males and females undergo has not been ascertained, nor has it been learned when copulation takes

place. The female is larger than the male and deposits only a single egg. In a well-ventilated insectary at Baton Rouge, during 1916, eggs were first noted on March 6 in vials in which the true sexes had first appeared 12 days before.

OVIPOSITION

The egg is often found resting in a small amount of white, cottony material secreted by the female. The true sexes apparently do not, as a rule, move far from their places of birth, eggs being found in the field in crevices on the trunks and limbs of poplar trees where living winged migrants (sexupara) and the dead bodies of others were present. In the insectary at Baton Rouge stem mothers were first seen on March 22, 1916, in vials where eggs had first been noted on March 6, giving a period of 16 days for the incubation of the egg.

FORMATION OF GALL

Of necessity the young, active stem mother (fundatrix), after issuing from the egg, must make its way to the developing leaves, where it settles down on the petiole and becomes responsible for the formation of a gall. While there is no absolute proof that such is the case, it is believed from field observations that one stem mother is responsible for one gall only and that a gall is only formed when a young stem mother locates on a leaf petiole.

SEASONAL HISTORY OF PEMPHIGUS POPULI-TRANSVERSUS IN BRIEF

The following summary has been prepared from observations made in the field and laboratory at Baton Rouge (fig. 1). The dates when the various stages appear and migration takes place probably depend to some extent upon the weather. It would be interesting to know the seasonal history of the species in the northern portion of its range where climatic conditions, especially as regards temperature, are so different from those existing in Louisiana.

The galls begin to develop on the petioles of the young leaves of *Populus deltoides* in the spring. They increase in size during the summer and by the time the leaves fall in the autumn some have reached a diameter of nearly an inch.

Of 1,175 leaves gathered from a poplar tree on July 26, 1916, 26.2 per cent had galls on their petioles. They occur on both small and large trees.

Winged migrants (fundatrigenia) have been found in the galls as early as June 1. The percentage of galls containing winged migrants, as well as the number found in anv one gall, increases as the season advances.

Winged migrants from the galls fly to various cruciferous plants. They have been found on the leaves of such plants as early as August 30 and as late as October 31.

The winged migrants give birth to viviparous females (virgogenia) which start colonies on the roots of crucifiers, upon which they feed.

The infestation at the roots of crucifiers, which is usually made apparent by the white, cottony material which the aphids excrete, gradually becomes more severe, owing to the increase in the number of the subterranean forms. It appears that, under certain conditions, the smaller, wingless viviparous females occurring in the soil are able to migrate to a considerable distance from their place of birth and there begin new colonies.

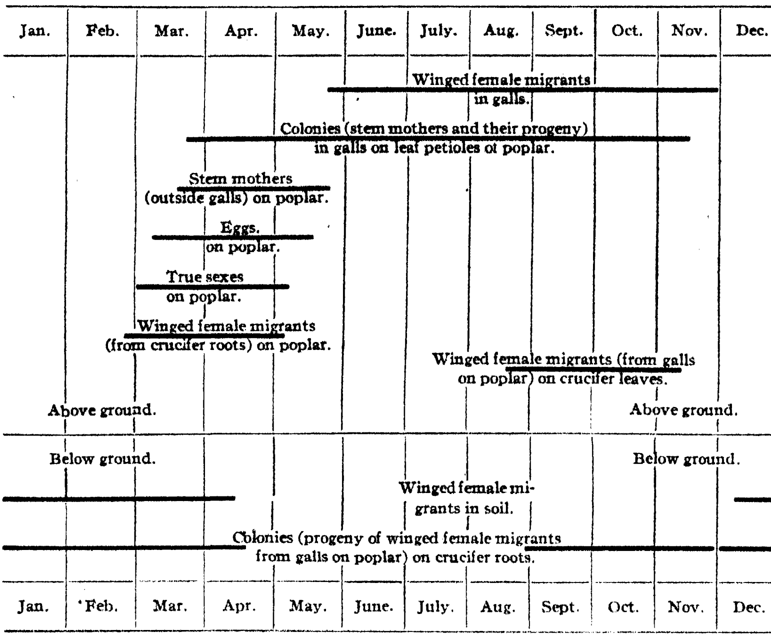

FIG. 1.—Diagram illustrating the seasonal history of *Pemphigus populi-transversus* at Baton Rouge, La.

Winged migrants (sexupara) appear in the subterranean colonies during the winter. They have been found at the roots of cabbage, turnip, Brussels sprouts, rape, *Coronopus didymus*, and *Roripa* sp. Colonies of a species of Pemphigus have also been found at the roots of mustard, cauliflower, broccoli, and *Lepidium virginicum*. No winged migrants were present in these colonies, but it appears probable that the aphids were of the same species.

In the spring the winged migrants fly from the crucifers to poplar trees where they give birth to the true sexes (sexuales), usually in crevices on the trunks and branches.

The sexed individuals take no food and, after pairing, the female deposits a single egg.

The stem mother (fundatrix), after issuing from the egg, makes its way to the young leaves of the poplar, where it settles down on a petiole. Here a gall begins to form about it.

DESCRIPTIONS OF STAGES OF PEMPHIGUS POPULI-TRANSVERSUS AND ITS GALL

By C. P. Gillette

Examples of this species in the collection of the Colorado Experiment Station, which were collected by Mr. J. T. Monell at St. Louis, Mo., on October 2, 1907, agree in every particular with Riley's description of the winged form, except that the fifth joint of the antenna is not quite as cylindrical as the description would indicate. Specimens sent by Mr. T. H. Jones, of the Bureau of Entomology, which were collected from similar galls at Baton Rouge, agree perfectly with the Missouri specimens, and with specimens taken at many different times in Colorado. Winged and wingless "lice" taken by Mr. E. S. Tucker, then of the Bureau of Entomology, from the roots of turnips at Baton Rouge, on March 6, 1915; by Mr. F. B. Paddock, State Entomologist, on turnips, at College Station, Tex., on February 13, 1914; and by Mr. T. H. Jones on Brussels sprouts at Baton Rouge, on March 4, 1916, agree well in structural details.

The galls are widely distributed over Colorado upon the broad-leaved cottonwoods; but they are not abundant, except upon an occasional tree. It should be stated here that the sexupara of this species is separated with some difficulty from the same form of *Pemphigus betae*. In the latter species, however, the permanent sensorium of joint 5 is of the normal form and never broad and irregular, inclosing chitinous islands, as in *populi-transversus*, and the spur is distinctly longer.

Fundatrix, first instar.—Described from specimens reared by Mr. T. H. Jones at Baton Rouge, La., and taken from the galls on March 3, 1916.

Ground color pale yellow tinged with green; head black; wax plates dusky; length, 0.60 mm.; width, 0.23 mm.; antenna, 0.45 mm., 4-jointed, joints 1, 2, and 3 subequal in length, joint 4 with spur, almost as long as 2 and 3 combined and very stout, and with several transverse rows of small chitinous points; all femora stout, the greatest width of the hind femur nearly equalling one-half its length; six longitudinal rows of wax plates upon the dorsum of segments 1 to 6 of the abdomen, and four rows of larger plates upon the dorsum of the thorax, each plate bearing at least one short, stout hair; legs and antennæ deep shining black with a few short stout gray hairs (Pl. 81, A).

Adult fundatrix.—Described from living specimens taken at Fort Collins, Colo., by Mr. L. C. Bragg, Assistant Entomologist of the Colorado Experiment Station, on September 22, 1916, from galls on leaves of *Populus deltoides*. The opening of the gall was a straight, transverse, or somewhat diagonal slit, passing from one-half to two-thirds of the way across the gall, but not a narrow and protruded mouthlike or liplike

opening. The galls at this date appeared to be fully grown. Besides the stem mother, there were, in each gall, a few winged "lice" quite dark in color, a good number of pupæ of varying sizes, the small ones being quite pale in color, and numerous small larvæ which were very light colored and heavily tufted with white waxy threads. The old gall mother seemed to be the sole parent of the gall colony, all of which normally acquire wings.

The stem mothers were a yellowish sordid green in color, very plump, covered with a fine white powder; head, the entire legs, including coxæ, and tips of the antennæ dusky to blackish; antenna 4-jointed and very short, not as long as hind tibia, in length approximately 0.40 mm.; length of body 2.50 to 3 mm.; joint 3 distinctly longer than joint 4 with spur, the proportion being about as 3 to 2; length of hind tibia 0.50 mm.; eyes very small. There are upon the dorsum six longitudinal rows of rather large wax plates beginning upon the mesothorax and extending to the seventh abdominal segment. Upon the prothorax and the eighth abdominal segment the number of plates is reduced to four (Pl. 81, C, H).

The writer also examined, in connection with this description, numerous specimens taken in Louisiana by Mr. T. H. Jones, in California by Messrs. E. Bethel and George P. Weldon, in Arizona by Mr. Bethel, and on the eastern and western slopes of the mountains in Colorado by Messrs. L. C. Bragg and C. P. Gillette.

It seems certain that the wingless stem mother that starts the gall of this species early in the season normally continues to feed and reproduce until the leaves mature in the fall, all of her young acquiring wings and going in search of the alternate food plants of the family Cruciferae.

FUNDATRIGENIA MIGRANT FROM THE GALLS.—In color and general appearance like the winged sexupara from turnips and Brussels sprouts. The specimens examined average about 0.25 mm. shorter in body length. The differences in the antennal segments are quite marked. Joint 3 has from two to six transverse sensoria, the usual number being three or four. Joint 4 is the shortest and weakest and rarely has a small sensorium. Joint 4 being somewhat smaller, and joint 5 slightly larger than in the sexupara, the contrast in size of these segments is very noticeable. The permanent sensoria on joints 5 and 6 are very large and irregular, and even may be cut into two by projecting chitinous margins. They always have upon their surfaces small chitinous pieces, one or two on joint 5 and two to four on joint 6, each bearing one or more short hairs. Upon joint 6 this large irregular sensorium may extend from the base of the spur to the middle of the segment and is nearly always very irregular in outline. The proportions of the segments are about as follows: 1, 21; 2, 30; 3, 66; 4, 31; 5, 39; 6 with spur, 68. There are many irregularities in the antennæ of this species, one of which is the frequent union of segments 3 and 4 into one (Pl. 81, J).

WINGLESS VIVIPAROUS FEMALE.—Described from specimens taken by Mr. T. H. Jones at Baton Rouge, La., from the roots of Brussels sprouts, on April 2, 1917.

General color sordid pale yellow, with head, antennæ, and legs dusky brown to blackish; tarsi and eyes black; length 2.50 mm.; width 1.60 mm.; antenna 0.45 mm., joints 3, and 5 plus spur, subequal; joint 4 much the shortest, being less than one-half as long as joint 3; beak barely attaining second coxæ; hind femora and tibiae

each 0.55 mm.; body and legs very free from hairs; apparently no wax glands on the body (Pl. 81, E, I).

PUPA.—Almost uniform pale lemon yellow with slight greenish tinge on abdomen and a shade of flesh color upon the thorax; wing pads very slightly dusky along the outer margins; head, antennæ, and all the legs dusky; eyes black.

WINGED SEXUPARA.—Described from living specimens taken by Mr. T. H. Jones, Baton Rouge, La., on March 21, 1917, bred from Brussels sprouts, and from preserved specimens from Mr. F. B. Paddock, College Station, Tex., which were taken on February 2, 1913; from Mr. E. S. Tucker, Baton Rouge, La., taken on March 5, 1915, on turnips; and from one specimen taken by Mr. L. C. Bragg, near Fort Collins, Colo., on watercress (*Roripa* sp.) on August 31, 1917.

Head, antenna, entire thorax above, mesothorax below, and entire legs black; wings slightly smoky, with subcostal vein black or blackish and heavy along the inner or lower margin of the stigma; abdomen sordid light greenish yellow without markings; body everywhere with a slight covering of gray powder; dorsum of abdomen covered more or less with a cottony secretion; length of body 2 mm.; wing 2.70 mm.; antenna 0.60 mm.; hind tibia 0.75 mm.; joints of antenna in following proportions: 1, 22; 2, 30; 3, 65; 4, 32; 5, 32; 6 with spur, 56; sensoria transverse, joint 3 with four to eight, usually five or six; 4 with two or three; 5 and 6, normally, with permanent sensoria only; spur near base of joint 3 distinct. Permanent sensorium on joint 5 usually very large, often inclosing one or two chitinous pieces as in fundatrigene. Nervures of wing dusky, the costal and subcostal being heavy and black; stigma blackish, nearly parallel sided, and about three times as long as broad (Pl. 81, D, K).

OVIPAROUS FEMALE.—Described from a number of specimens deposited in a cage in the laboratory by specimens sent by Mr. T. H. Jones, from Baton Rouge, La.

General color buttercup yellow, with head, antennæ, and legs whitish and very transparent; a little dusky on the vertex; eyes black; antennæ short, 0.15 mm. long, 4-jointed, joints subequal, the first, and last including the spur, longest; length of body 0.70 to 0.90 mm. (Pl. 81, G).

EGG.—Oblong oval, glistening, varying in color from dull white to yellow. Ten eggs, deposited in the laboratory, averaged 0.54 mm. in length, ranging from 0.48 to 0.57 mm., and 0.21 mm. in width, ranging from 0.19 to 0.24 mm.

MALE.—Described from specimens born along with the oviparous females.

The males differ from the females by being pale yellowish green in color, more slender in form, and a little shorter, 0.60 to 0.65 mm. long, the legs very stout (Pl. 81, F).

GALLS.—Riley described the gall of this species as follows:

Formed upon the petiole near the base of the leaf of *Populus monilifera* and *P. balsamifera*. An elongate-oval swelling, causing the curving and broadening of the petiole, and opening on the opposite side by a transverse slit, with a whitish, slightly thickened, and elevated margin, recalling human lips.

The writer has studied large numbers of galls of this species from Colorado and Louisiana, the latter collected by Mr. T. H. Jones. When fully grown, they vary normally from about 12 to 18 mm., extra sizes attaining 20 or even 25 mm. in their greatest diameter, which is usually

in the direction of the petiole of the leaf. The fundatrix, or stem mother, soon after hatching from the egg, locates upon the petiole of a very young opening leaf, causing it to curve and thicken, and form a transverse groove (Pl. 81, B, a, b, c) at the point of attack. The petiole continues to thicken, and the groove to deepen, forming a pit or groove which carries the "louse" with it, and the two margins or lips gradually meet, inclosing the "louse" in a spacious cavity. The mouth or slit is usually transverse, but may be turned more or less in a vertical position, and the margins may, or may not, be thickened or protruded By the time the inmates become mature and ready to fly, the lips separate enough to allow the "lice" to pass out in search of the alternate food plants. The expanded petiole of the leaf can be easily seen extending along the convex surface of the gall opposite the mouthlike opening. (For typical forms of this gall, see Pl. 82; 84, A–E.)

The gall of this species is readily separated from the galls of *Pemphigus populicaulis*, which have a long curved opening formed by the twisting of the petiole upon itself, or from the galls of *P. populi-ramulorum*, which develop upon the side of tender growing twigs (Pl. 84, A–E).

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[1] More recently the writer has learned that as early as 1902 Dr. F. H. Chittenden recorded the receipt of specimens of an unknown species of Pemphigus from Texas. These were sent by Mr. S. A. McHenry, of the Beeville Substation of the Texas Experiment Stations, on February 14, 1901, with the information that the species was doing injury to the roots of cabbage in the vicinity of Beeville, some of the fields being reported as totally destroyed. (CHITTENDEN, F. H. SOME INSECTS INJURIOUS TO VEGETABLE CROPS. U. S. Dept. Agr., Div. Ent., Bul. 33, n. s., p. 79. 1902.)

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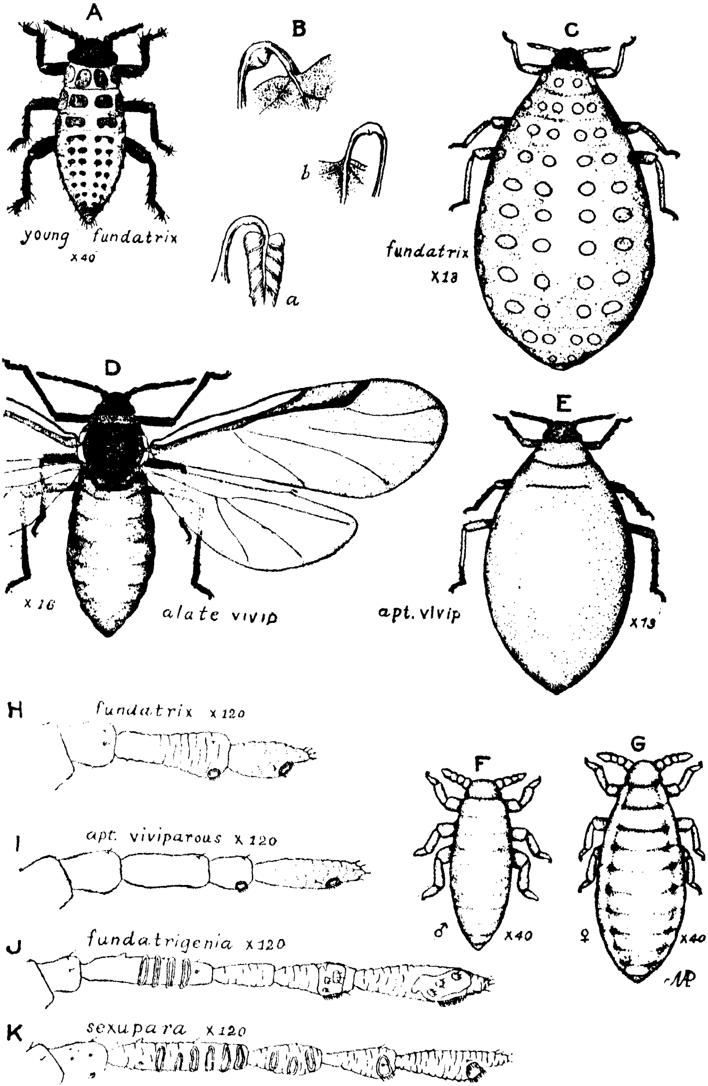
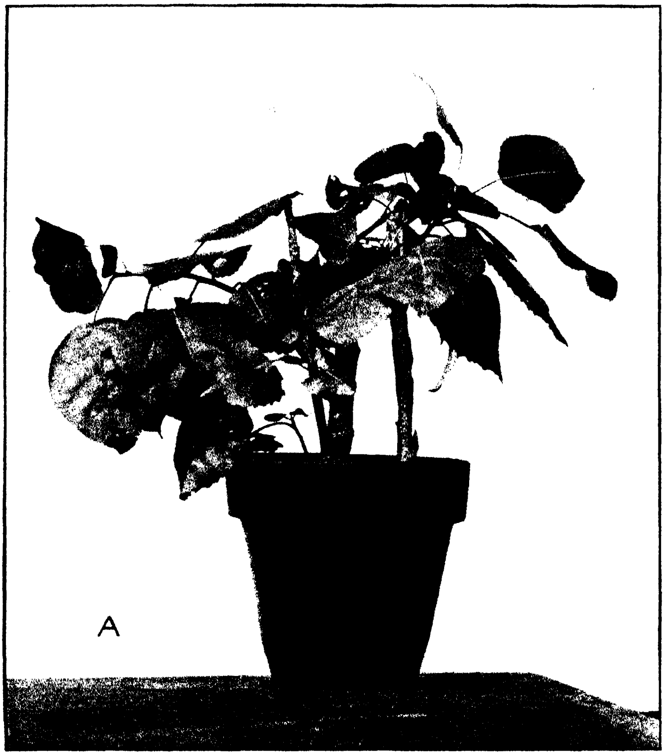
PLATE 81

Pemphigus populi-transversus:

A.—Young fundatrix, first instar. ×40.
B, a. b, c.—Beginning of galls on cottonwood leaves.
C.—Adult fundatrix with cottony secretion removed. ×13.
D.—Winged sexupara from roots of Brussels sprouts. ×16.
E.—Wingless virgogene from roots of Brussels sprouts. ×13.
F.—Male. ×40.
G.—Oviparous female. ×40.
H.—Antenna of fundatrix. ×120.
I.—Antenna of wingless viviparous female from Brussels sprouts. ×120.
J.—Antenna of winged fundatrigenia from gall. ×120.
K.—Antenna of winged sexupara from Brussels sprouts. ×120.
Drawn by Miss Miriam A. Palmer.


A
young fundatrix
X40
B
b
a
C
fundatrix
X13
D
X16
alate vivip
E
apt. vivip
X13
H
fundatrix X120
I
apt. viviparous X120
J
fundatrigenia X120
K
sexupara X120
F
♂
X40
G
♀
X40


A

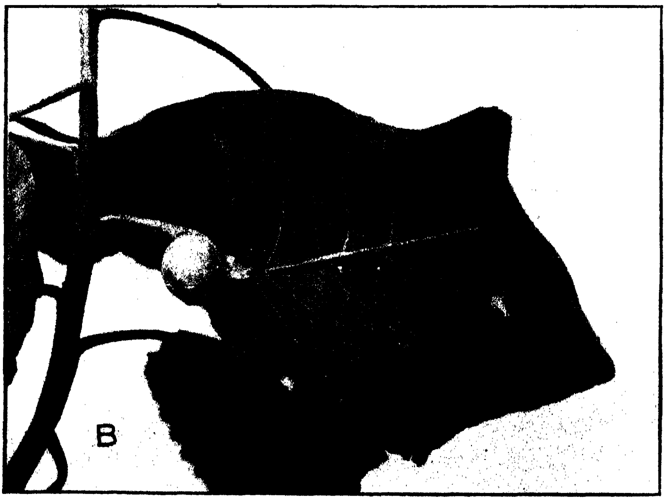

B

PLATE 82

Pemphigus populi-transversus:

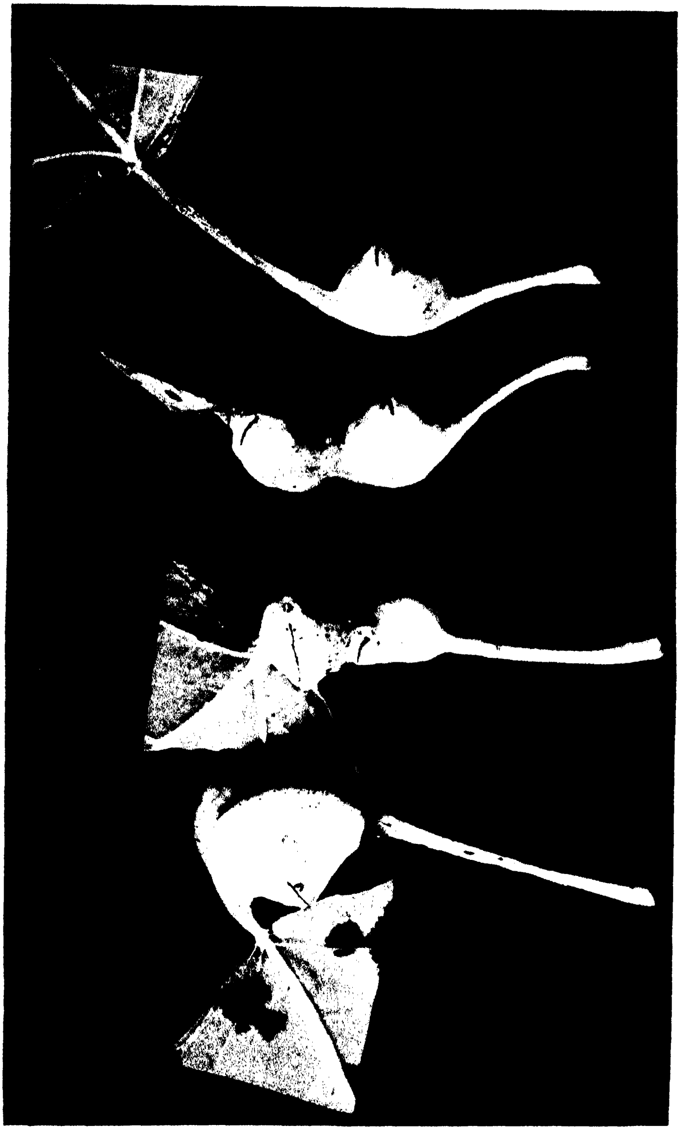
A.—Gall on poplar cutting. Cuttings were collected before the buds began to swell and were planted in sand in flowerpots kept in a greenhouse at Baton Rouge, La. Young stem mothers of *Pemphigus populi-transversus*, obtained indirectly from winged female migrants from roots of crucifers, were placed on growing cuttings on March 23, 1916. The photograph, taken on May 27, shows pot containing cuttings. One developing gall can be seen on twig at upper left.

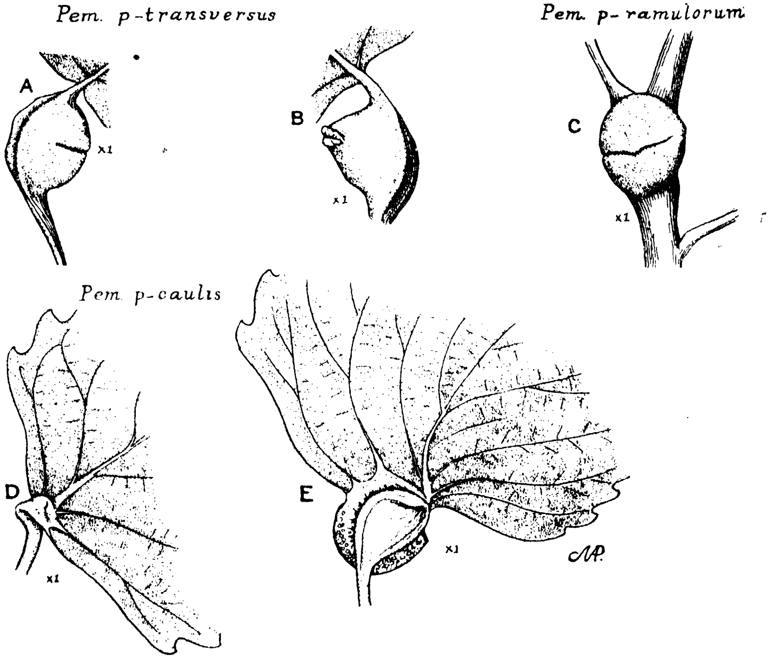
B.—Gall shown in A, enlarged to nearly natural size. Beside it can be seen the slit of a small gall which has failed to develop to any considerable extent.

PLATE 83

Pemphigus populi-transversus:

Variation in size of galls and location on leaf petioles of *Populus deltoides*, Baton Rouge, La., September 15, 1916. About natural size. Shows lips of galls all protruding, and in some cases thickened.




Pem. p-transversus
Pem. p-ramulorum
A
x1
B
x1
C
x1
Pem. p-caulis
D
x1
E
x1

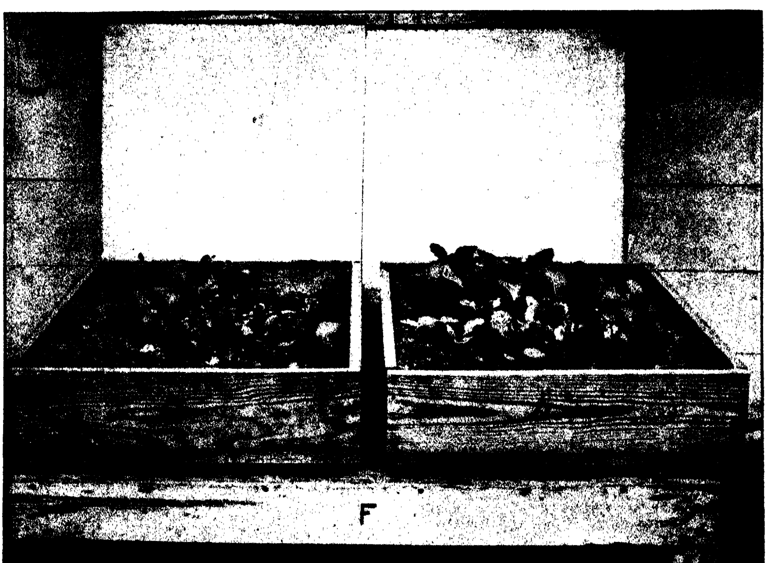

F

PLATE 84

A.—Gall of *Pemphigus populi-transversus*, lips not protruding.

B.—Gall of *Pemphigus populi-transversus*, lips protruding.

C.—Gall of *Pemphigus populi-ramulorum.*

D.—Beginning of gall of *Pemphigus populicaulis.*

E.—Full-grown gall of *Pemphigus populicaulis.*

All natural size. Drawn by Miriam A. Palmer.

F.—Turnip seedlings, showing injury by *Pemphigus populi-transversus: a*, infested; *b*, control. Turnips were planted at Baton Rouge, La., on September 16, 1916, in boxes having cloth-covered tops. Galls of *P. populi-transversus* were placed in box a on September 28. Photographed on October 13, at which time the wingless form of the aphid was abundant on roots of plants in box a. No root aphids found in box b. Note difference in growth of plants in the two boxes.

PLATE 85

Pemphigus populi-transversus:

F.—White cottony secretion at roots of Brussels sprouts due to presence of *Pemphigus populi-transversus*. Photographed at Baton Rouge, La., on February 10, 1916.

STEM LESIONS CAUSED BY EXCESSIVE HEAT

By Carl Hartley

Pathologist, Investigations in Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture

WHITESPOT INJURY

During work on the damping-off disease of pines (*Pinus* spp.) in 1909 the writer noticed in a nursery at Halsey, in the Nebraska sand hills, a type of disease closely corresponding to the old published descriptions of damping-off. The stems of very young seedlings developed shrunken areas at the soil surface. Commonly the entire stem was constricted by the lesion, the seedling fell over, and died. The writer has previously referred to this type of injury as "whitespot" (*4, p. 5*).[1] Close examination showed that this trouble differed in several ways from the type of damping-off which the writer and assistants have produced by inoculation with common pine-seedling parasites (*Pythium debaryanum* Hesse, *Corticium vagum* B. and C., and species of Fusarium). The primary whitespot lesions were in all cases limited to the stems, and usually just above the ground line. The whitespot lesion is very light in color, and this characteristic color continues to the very edge, making a sharp line of demarcation from the healthy tissue. Lesions may continue definitely limited for some days, and the upper stem and cotyledons remain turgid. In this early stage most cases of whitespot injury are easily distinguished from damping-off. Typical damping-off in porous soils is primarily a rootrot, which may attack above the ground line, but which more commonly attacks below. Damping-off lesions caused by any of the above-mentioned fungi or by *Botrytis cinerea* vary in color at different stages, gradually shading into the tissue still unaffected, and progress continuously both upward and downward.

This whitespot injury was at first supposed to be merely a special type of damping-off. Cultures made from the whitespot lesions failed to develop regularly any recognizable parasites, while most of the parallel cultures from lesions of the rootrot type yielded *Pythium debaryanum*. *Alternaria* sp. was the only fungus commonly obtained from the white spots.

Further examination showed that, in cases where whitespot lesions affected one side of the stem only, it was nearly always the south or southwest side. On seedlings which had been girdled, the lesions, if at all asymmetrical, extended higher on the south than on the north side. The south margins of the seed beds, imperfectly protected by the shade

[1] Reference is made by number (italic) to "Literature cited," pp. 603–604.

frames, contained more whitespot lesions than other parts of the beds. In a single bed left entirely without shade, most of the seedlings died from whitespot. These observations indicated insolation as at least a contributory cause of whitespot injury. The nursery practice involved as little watering as possible during the damping-off period. In plots given somewhat more frequent watering than the general beds, subsequent counts showed only three-fifths as much whitespot injury as elsewhere. During the three succeeding years, the nurserymen gave the seed beds much more frequent watering and more careful shading than in 1909. In careful examinations during these three years only occasional cases of whitespot were found.

The foregoing data all pointed to a physical rather than a parasitic cause for whitespot injury. Heat and light were the physical factors toward which suspicion was directed. The temperature in the surface layer of soil in the seed beds, even under the half-shade of the lath frame, was found to go as high as 52° C. The apparent preventive effect of frequent watering was believed to be due to the lowering of the soil temperature.

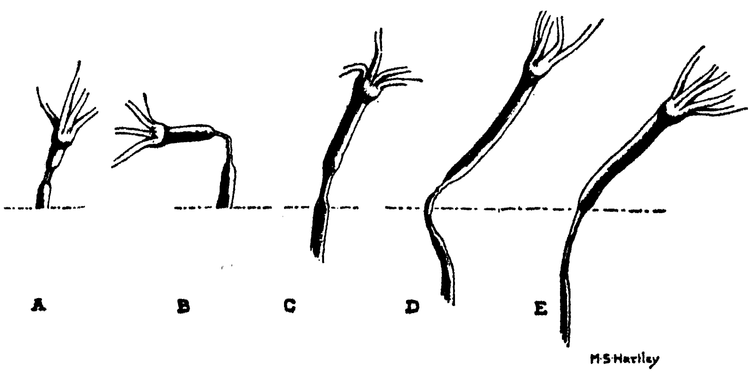


FIG. 1.—Lesions on seedlings of *Pinus ponderosa:* Seedlings A and D were injured by the sun's rays condensed by a lens. B was injured by a hot wire, C by an incandescent lamp, and E by the direct sun. The leaves remained turgid for 5 to 11 days after the lesions were produced. The horizontal line indicates the location of the soil surface at the time the seedlings were subjected to heat. Natural size.

In tests conducted at Washington, D. C., with seedlings of *Pinus ponderosa* typical whitespot lesions have been artificially produced. Five representative experiments are described in the following paragraphs and are illustrated in figure 1.

SEEDLING A.—The stem was subjected for less than two minutes to sunlight passed through a condensing lens at the point indicated by the constriction. The stem immediately collapsed and bent over at the point of the lesion. The soil around the root at the time was dry, and the seedling was distinctly wilted before it was heated. Later the pot was watered, and the seedling restored to a nearly vertical condition by propping. Turgor returned and was maintained for a week, but was followed by the decay of the root and wilting of the plant without extension of the original lesion (fig. 1, A).

SEEDLING B.—A heated wire held 1.5 mm. to the right of the stem for five minutes resulted in the lesion shown in figure 1, B. The plant remained turgid for 11 days and a whorl of new leaves appeared. The stem at the soil surface then decayed, apparently from a root infection, bearing a mat of pink spore masses.

SEEDLING C.—A Mazda incandescent lamp placed beside and slightly above the seedling for nearly two hours killed the tips of the cotyledons and produced a lesion just above the soil surface, without perceptible injury to intermediate tissue. In its location this lesion was thoroughly typical of those commonly found in the seed beds. Five days later fungus hyphæ appeared on the lesion, and the stem broke over at that point. The lesion then progressed both up the stem and into the root, and the seedling wilted. Spores of *Alternaria* spp. were promptly produced on placing the seedling in moist chamber (fig. 1, C).

SEEDLING D.—The stem and surrounding soil were subjected for three minutes to sunlight through a condensing lens, striking both the stem and soil surface at angles of about 45°. The resulting lesion extended about 3 mm. below the soil surface and 4 mm. above it. Mechanical support was required to keep the seedling from falling. Hyphæ soon appeared on the lesion at the soil surface, and in eight days after treatment wilting occurred, a species of Alternaria fruiting on the lesion in moist chamber (fig. 1, D).

SEEDLING E.—On a hot day, seedlings planted in loam in a 3-inch pot were placed immediately south of a brick wall. At night the seedling shown in figure 1, E, and another seedling were leaning slightly, but were apparently uninjured. An examination of the underground parts showed that these were distinctly shriveled from the soil surface to a point 10 mm. below; a third seedling in the pot, still erect and normal in appearance, was also constricted just below the soil surface. The plants were repotted and kept under observation. The tops of all remained entirely healthy for several days. At length the lesion began to extend up the stem, and on the eighth day wilting occurred. The entire root and 10 mm. of the stem above the soil line had become involved in the original lesion. Spores of species of Dactylosporium, Alternaria, and Fusarium appeared on the lesion in moist chamber.

Numerous seedlings of the same original lot were kept in the same room as the seedlings listed above during the period of the tests. None developed lesions which could have been mistaken for whitespot.

In all of the seedlings whose stems were heated directly the lesions were at first a dark grayish green, changing in 24 hours to the light color and shriveled appearance characteristic of whitespot lesions on seedlings in the nurseries. The immediate darkening is supposed to be due to the filling of the intercellular spaces with cell sap, while the ultimate light color presumably indicates the loss of liquid from both the intercellular spaces and the lumina of the cells and its replacement by air. In all cases the lesions remained definitely limited for several days, and were then extended, apparently as a result of infection by fungi not commonly capable of attacking uninjured plants. It appeared that in most cases neither the heat nor the fungi later entering the lesions stopped conduction or evolved toxins in sufficient quantity to cause the death of the leaves, as reported for another plant by Overton (*11*). Wilting finally occurred, it is believed, only when fungi entering at the lesion or at some point below it had penetrated the absorbing portion of the root. It may be remarked that in many cases of damping-off caused by the

ordinary seedling parasites, wilting probably occurs only after the absorbing portions of the root are invaded by the parasite.

The limitation of the whitespot lesions to the stem just above the soil surface in seedling C and in most of those observed in the nurseries, indicates that the combined radiation from the heated soil and from the sun direct ordinarily results in a temperature in the stem at that point higher than the temperature in the surface soil. In an experiment not described above, in which the source of heat was directly above the seedling, the lesion was, as might be expected, just below the soil surface rather than above it. The pot containing seedling E was also so placed that the sun's rays were more nearly perpendicular to the soil surface than in level seed beds, with the same result. It is probable that in at least some cases heat lesions will occur in the nurseries partly or entirely below the soil surface, as in seedlings D and E. It will be impossible, by any ordinary method of field observation, to distinguish from damping-off cases such as that of seedling E. Both the angle of the sun's rays, and the absorbing, conducting, and radiating capacity of the soil and of the stem will, of course, help determine whether the stem will be hottest above or just below the soil surface.

Münch (*8*, *9*) has described the same type of injury to tree seedlings in Germany, attributing it positively to heat at the soil surface. With a thermometer having a thin, flat bulb he obtained very high temperatures in the surface soil (*10*). Others have also reported surface temperatures in unprotected soil from 55° to 68° C., or even higher (*2*, *p. 55; 7*, *p. 13; 12*; *17*). Münch made an incubator test in which coniferous seedlings survived for two to three hours at temperatures not exceeding 52°, but were killed by maxima of 54° to 55° C. This seems in general to agree with the temperatures reported as being fatal to most growing plants.

Typical whitespot injury to seedlings has been found in several different States, though nowhere has it been observed to cause as heavy losses as at the Nebraska nursery, where it was first seen. Whitespot is not limited to conifers. In the vicinity of the Nebraska nursery an examination of fields of rye (*Secale cereale*) and cowpeas (*Vigna sinensis*) showed that both were affected in the seedling stage in much the same way as the pines. In the cowpeas the localization of the white, constricted lesions just above the soil surface was very marked. Plate cultures yielded no fungus suspected of parasitism. The rye seedlings were affected in the same way, though constriction was less in evidence than in the more fleshy plants. The relation between the disease and exposure to sun was very evident in the case of the rye. In the level portion of the field a moderate proportion, perhaps 5 per cent, of the shoots were affected. Where a dead furrow crossed the field from east to west this uniform distribution of disease was broken. On the wall of the dead furrow having a north exposure the disease was not noticeable.

On the other wall, exposed to the south, the percentage of affected shoots was much greater than on the level surface of the rest of the field. Wind action was not excluded as a possible cause of the cowpea lesions, but the protected location of the nursery, so far as south winds are concerned, made the evidence rather conclusive that insolation rather than wind was responsible for the lesions on the rye. Münch (*8*) reports whitespot on maple, vetch, and peas, and believes that in some cases germinating seeds, as well as seedlings which have already broken soil, are killed by overheated soil.

Whitespot is not always fatal, even when the lesion girdles the stem. Two seedlings of *Pinus ponderosa* which had been girdled by definite whitespot lesions, slightly shrunken but not severe enough to cause breaking over, were marked for later observation. At the end of the season the lesions had disappeared and the plants seemed in every way normal. In leaf lesions due to heat, Sorauer (*15, p. 638*) has after several weeks observed a regeneration of chloroplasts in slightly affected tissues.

All things considered, whitespot lesions are believed to be caused mainly by excessive heat. While light as such may possibly take part in some cases, it evidently does not enter into all cases of injury. The relative unimportance of light as distinguished from heat is indicated by the numerous lesions under slat frames, the extension of all serious lesions to the north sides of stems, and the experimental production of lesions below the soil surface (seedlings D, E, and others). The preliminary experiments here reported were mostly at excessive temperatures, and absolute proof that heat alone is the cause of the common lesions in the seed beds must await further experiments at temperatures which more commonly occur in nature.

BASAL LESIONS ON SEEDLINGS SEVERAL MONTHS OLD

A type of trouble which is probably related to the whitespot described in the foregoing was observed in 1915 in the seed beds of a nursery of the United States Forest Service, located at an elevation of 7,300 feet in the Wasatch Mountains, Utah. The plants affected were spruce and Douglas fir which had been raised from seed the preceding year. They had made a normal height growth during their first season and remained green throughout the winter under a heavy coating of snow which covered them for more than five months. Two or three weeks after the snow melted many of the seedlings began to turn yellow and ultimately died. Examination showed dead bark, beginning at the soil surface and extending up the stem from 3 to 9 mm. In many cases the lesion extended farther up the stem on the south than on the north side, and on some seedlings lesions were found which were entirely limited to the south side of the stem, and had started to heal over from the edges. In no case was there found any such swelling above the lesion as occurs above stem-girdle lesions on older stock. In many of the advanced cases the cortex

from the base of the stem was partly or entirely gone. Careful examination, however, indicated that even these lesions could not be attributed to any biting insect. The affected seedlings were distributed rather evenly over the beds, but in no case were any diseased plants found immediately north of posts, or on the north exposed slopes at the ends of beds, where the seedlings were somewhat protected from the sun during the hottest part of the day. Spruce, a more shade-loving tree than Douglas fir, also suffered more from the disease. The observations made indicate that the death of these seedlings was due to whitespot lesions occurring during the latter part of the preceding summer. The altitude of the nursery at first thought renders it improbable that excessive heat should have been concerned in causing the injury. While, of course, the temperature of the air at such elevations is never very high, the heat of rocks and gravel exposed to the sun at high altitudes is well known. Tubeuf (*17*) reports a surface soil temperature of 60° C. (140° F.) at an elevation of 10,000 feet in Yellowstone Park, 200 miles directly north of the Wasatch region.

BASAL STEM-GIRDLE ON OLDER STOCK

Münch and others (*5, 7, p. 13; 12, 13, p. 397; 14, 15, p. 638*) have further attributed to excessive surface temperatures of the soil the "*Einschnürungskrankheit,*" or stem-girdle, of older nursery stock or young forest trees of both conifers and broad-leaved trees. Dr. B. T. Galloway, of the Bureau of Plant Industry, told the writer that he had found basal lesions on young willows at Chico, Cal., which he attributed to excessive heat. In conifers this disease involves death of the base of the stem of 2-to-4-year-old seedlings and transplants. Lesions are definitely limited, and the swollen growth of the stem just above the lesion, which results from the girdling and interference with food movement, gives an appearance of constriction at the lesion itself. This disease is figured by Tubeuf (*16, p. 492*) and ascribed to *Pestalozzia hartigii*. Hartig (*3*) had originally ascribed it to the freezing of thin pools of water standing on the surface of the beds. The parasitism of *P. hartigii* has failed of confirmation (*1*), and Tubeuf (*17*) now seems to favor the view of Münch, that heat of the soil is responsible. This disease has been found at widely separated points in the United States. The writer has seen what appeared to be stem-girdle on two species of the white and three of the pitch pines, two spruces, *Abies concolor* (Gord) Parry, *Pseudotsuga taxifolia* (Poir.) Brit. *Thuja* sp., and *Juniperus* sp.[1] Its appearance and its ability to attack representatives of so many different genera favor a nonparasitic diagnosis. The thicker cortical tissues of the woody stems should make the cambium slower to reach maximum temperature, and prevent quite as high a temperature being reached, as in the case of the younger stems,

[1] Several of these observations as to coniferous species affected were communicated by Dr. J. V. Hoffman, of the Forest Service, and confirmed by the writer's examination of his specimens.

which are subject to whitespot. It is worthy of note that no cases of stem girdle have been found in four seasons' examinations of the Nebraska nursery at which whitespot has been so frequent. The heat hypothesis nevertheless seems the best explanation of stem-girdle so far offered. Tubeuf's experiments with warm water (*18*) are of interest in this connection as indicating that moderately high, long-continued temperatures do not necessarily kill simply by their drying effect, as has sometimes been claimed.

LESIONS ON UPPER PARTS OF STEMS

Older 2-needled pines and herbaceous plans as well have been observed by the writer to develop typical shrunken, definitely limited whitespot lesions on young growth of the upper parts of their stems, usually at points where an abnormal bend had made the surface nearly perpendicular to the sun's rays. Such lesions seldom girdle stems, and are rarely, if ever, of economic importance.

In the case of *Pinus strobus* the unusual amount of attention which pathologists have given it in the last two years has resulted in the finding by blister-rust scouts at five different places of yellowish lesions on young stems, sunken, and in all or nearly all cases limited to one side of the stem. Sections made by Dr. R. H. Colley, of the Bureau of Plant Industry, showed a collapsed condition of the tissues, but with absence of mycelium. Observations on these lesions in northern Wisconsin by Mr. R. G. Pierce, of the Bureau of Plant Industry, showed that practically all occur on the upper sides of bent shoots or on the west sides of vertical shoots, though a single case was found on the north side. The greater number of the affected plants were on the west sides of the nursery beds.

Most of these lesions are presumed to be due to heat. In the few cases in which soft young shoots of *Pinus banksiana* have been found girdled and bent over at the point of the lesion, mechanical bending is also suggested as a possible cause.

LESIONS DUE TO EXCESSIVE BENDING

Following cold weather with high winds, pine seedlings 1 to 2 weeks old have been found with white constricted basal lesions in some ways not like those produced by heat. There is reason to believe that these are due to the constant bending in high wind, at length causing the collapse of the cortical tissues without the stress being sufficient at any one time to rupture the epidermis or break the fibrovascular bundles. This is apparently an analogous case to the death of tissue between the veins of sugar maple leaves exposed to storm (*6, p. 27–28*). A few cases of lesions on shoots of older pines have already been mentioned as possibly the results of mechanical bending rather than of heat, and it is entirely possible that the whitespot lesions observed on cowpea seedlings should

be attributed to bending rather than to heat. Basal stem-girdle lesions found on 4-months-old wild olive seedlings (*Elaeagnus* sp.) and characterized by very slight vertical extension may be due to excessive bending. However, stem lesions caused by bending without breakage are not believed to be common enough to give rise to serious confusion with those caused by heat.

PREVENTIVE MEASURES

Assuming the correctness of the hypothesis that most of them are caused by heat, the logical procedure for preventing whitespot and the basal lesions on older stems is to avoid soils especially liable to overheating, and in established nurseries where trouble occurs, to artificially prevent heating. Soil with loose texture or dark surface is presumed most likely to overheat at the surface. Shading and frequent light watering have already been found helpful in preventing whitespot. Encouraging free air movement and artificially compacting the soil to increase its conducting capacity have been suggested as having prophylactic value.

SUMMARY

(1) Very young seedlings of conifers and certain other plants were found dying in large numbers in a Nebraska nursery from a disease which, because of its characteristic lesions, the writer has called "whitespot." The trouble has been found, though less commonly, in other localities. It is distinct from the common damping-off disease, but resembles it so closely that it is very likely to be confused with it. The lesions do not seriously interfere with the upward movement of water.

(2) The location of the whitespot lesions on the stems, their observed relation to insolation and to dry surface soil, and the production of typical lesions by artificial heating, indicate excessive heat as the cause of most of the whitespot trouble.

(3) The observation in the surface soil in the seed beds in question, and by other investigators in other places, of temperatures well over 50° C., with reported maxima as high as 68°, further substantiates the hypothesis that whitespot is due to excessive heat.

(4) Killing lesions on stems of older conifers ranging in age from several months to 4 years, are also attributed to heat. The causal importance of heat in these lesions on woody or semiwoody stems is less well established than in the case of whitespot. Further experimental work at temperatures such as actually occur in nature is necessary to settle finally the pathological importance of high soil temperature.

(5) Lesions involving young cortex and resembling those attributed to heat are probably in some cases caused by repeated bending in heavy wind without visible breakage. These are believed to be too rare to give rise to serious confusion.

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WORK AND PARASITISM OF THE MEDITERRANEAN FRUIT FLY IN HAWAII DURING 1917

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The interesting history of the many successful introductions of beneficial insects into Hawaii includes little exact data on the activities of these insects during the first few years after liberation. It is during this period that some immediate adaptations may be necessary to enable the insects to conform to a new environment, and many unexpected fluctuations may occur between the various species introduced to attack the same host before a balance is reached among them that can be expected to remain fairly constant during the years to follow. This period, then, is of much biologic interest. Apart from this, insufficient data have been published which convey accurate information concerning the work of these insects and the enormous check constantly being exerted by them over destructive pests, without which many forms of agriculture could not be conducted with profit. Aside from the aid which the entomologist can give the farmer in distributing beneficial insects over the earth beyond their natural barriers, it is his duty to obtain informing data on the kind and extent of assistance that is continually being rendered to agriculture through the work of beneficial insects, both native and introduced.

The spread and value of the parasites introduced into Hawaii to attack the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) have been watched and recorded yearly since the first liberations in 1913.[1] With the object of continuing this unbroken series of data and of again informing those interested of the degree of success attending these introductions, the following data are given to indicate the work of the established parasites throughout the year 1917, and the extent of fruit-fly injury caused to fruits in the Territory during that year.

During the year there was a rather heavy infestation of several varieties of fruits, some kinds being badly infested, as shown in Table I. From 913 peaches (*Amygdalus persica*) collected about Honolulu a total of 13,904 larvæ developed, or an average of 15.2 maggots to each fruit. Hardly a peach was sufficiently free from maggots to be edible. From

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17,960 kamani nuts (*Terminalia catappa*) collected during the year, 143,246 maggots were obtained, or an average of 8 to each fruit. Fortunately most other fruits were somewhat less infested than these. Though the infestation is considerable, the parasitism, as shown in Tables II and III, is very high in some cases, and during the year averaged 47.5 per cent for all fruits taken together, as determined from records on 72,139 larvæ.

TABLE I.—*Extent of infestation of host fruits by larvæ of Ceratitis capitata in Hawaii during 1917*

Host fruit.	Number of fruits collected.	Number of *C. capitata* larvæ emerging.	Average number of larvæ per fruit in 1917.	Average number of larvæ per fruit in 1916.
Indian almond (*Terminalia catappa*)	17,960	143,246	8.0	9.5
Mango (*Mangifera indica*)	648	5,250	8.1	1.7
Coffee (*Coffea arabica*)	58,272	45,788	.8	.5
Strawberry guava (*Psidium cattleianum*)	9,872	19,795	2.0	1.6
Black myrobalan (*Terminalia chebula*)	1,767	10,411	5.9	7.0
Peach (*Amygdalus persica*)	913	13,904	15.2	20.5
Rose-apple (*Eugenia jambos*)	1,115	9,811	8.8	5.5
Satin-leaf (*Chrysophyllum olivaeforme*)	6,905	23,745	3.4	2.0
French cherry (*Eugenia uniflora*)	21,554	21,911	1.0	.8
West Indian medlar (*Mimusops elengi*)	4,133	7,287	1.8	5.3
Yellow-wood (*Ochrosia elliptica*)	56	1,312	23.5	3.1
Kamani (*Calophyllum inophyllum*)	346	838	2.4	3.3
Yellow oleander (*Thevetia neriifolia*)	2,960	16,667	5.7	3.6
Carambola (*Averrhoa carambola*)	316	185	.6	1.3
Chinese orange (*Citrus japonica*)	8,050	14,090	1.8	3.1
Guava (*Psidium guajava*)	1,342	6,009	4.5	6.8
Loquat (*Eriobotrya japonica*)	1,980	5,204	2.6	....
Kona orange (*Citrus sinensis*)	350	1,600	4.6	....
Mandarin orange (*Citrus nobilis deliciosa*)	341	732	2.2	....
Sapodilla (*Achras sapota*)	104	488	4.7	....
White sapote (*Casimiroa edulis*)	244	1,845	7.6	....
Wampi (*Clausena wampi*)	117	24	.2	....

TABLE II.—*Percentage of larval parasitism of Ceratitis capitata in Hawaii in 1917* [a]

Host fruit.	Month of collection.	Number of larvæ emerging during first 2–6 days.	Percentage of parasitism.				
			Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
	1917.						
Indian almond	January	677	7.5	44.3	....	1.3	53.6
Do	February	690	6.1	16.8	....	1.3	24.2
Do	March	302	10.3	22.2	....	7.3	39.8
Do	April	342	10.2	22.8	....	3.5	36.5
Do	May	1,035	11.4	34.3	0.3	.7	46.7
Do	August	4,620	4.4	15.8	.2	3.5	23.9
Do	September	5,148	5.0	46.8	.02	3.3	55.12

[a] Most of the fruits represented in this table were collected about Honolulu at low elevations. The coffee, however, was collected on the island of Hawaii, in addition to localities in Honolulu, and much of it came from points from 1,000 to 2,000 feet above sea level. The March collection of coffee came entirely from Kona on the island of Hawaii.

TABLE II.—*Percentage of larval parasitism of Ceratitis capitata in Hawaii in 1917*—Con.

Host fruit.	Month of collection.	Number of larvæ emerging during first 2–6 days.	Percentage of parasitism.				
			Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
	1917.						
Indian almond	October	6,149	12.1	29.6	.3	15.2	57.2
Do	November	3,791	12.1	31.5	.5	30.3	74.4
Do	December	961	11.0	32.3	2.5	19.1	64.9
Mango	February						
Do	June	567	2.8	14.6	.7	8.0	26.1
Do	July	207	3.9	15.0	2.9	12.6	34.4
Do	August	35			8.6	2.9	11.5
Coffee[a]	January	3,638	59.3	10.6	.8		70.7
Do	February	501	72.4	6.6	4.4		83.4
Do	March	1,722	50.0	33.2	5.9		89.1
Do	June	554	55.4	1.4			56.8
Do	August	22		9.1	45.5		54.6
Do	September	3,527	5.9	11.9	33.2	.03	51.03
Do	October	2,196	3.4	1.0	66.1	.05	70.55
Do	November	1,541	35.0	5.9	38.4	.5	79.8
Do	December	176	21.0	3.4	24.4	.6	49.4
Strawberry guava	January	18	16.7	33.3			50.0
Do	March	804	20.0	30.0	7.6	.1	57.7
Do	April	1,172	13.7	39.8	3.2	1.1	57.8
Do	May	603	12.3	50.7	1.7	1.8	66.5
Do	June	1,034	10.9	31.9	1.1	4.4	48.2
Do	July	939	6.1	74.4		.9	81.1
Do	August	220	11.8	15.5	23.6	12.3	63.2
Do	November	160		21.3	6.9	30.6	58.8
Do	December	267		57.7	3.4	4.1	65.2
Black myrobalan	January	355	8.2	5.6	4.2	34.6	52.6
Do	October	2,860	.9	.7	.5	3.9	6.0
Peach	April	263	.8	47.9		2.3	51.0
Do	May	833	3.6	37.8		9.2	51.5
Do	June	2,343	8.1	17.0		14.5	39.6
Do	July	1,391	6.3	18.0		44.8	69.1
Rose-apple	May	1,021	8.0	13.6		.1	21.7
Do	June	386	1.6	20.7		1.3	23.6
Do	July	274	.7	27.7		1.8	30.2
Do	August	358	.6	29.1		1.1	30.8
Satin-leaf	February	2,484	18.4	3.4	1.3	.9	24.0
Do	March	831	37.5	5.4	10.7	1.1	54.7
French cherry	January	1,258	13.4	19.5	5.9	.8	39.6
Do	February	290	13.8	31.4	3.8	1.4	50.4
Do	March	1,320	13.7	23.8	12.3	.5	50.3
Do	April	351	5.1	24.8	33.0		62.9
Do	May	346	6.7	9.5	39.0	.6	55.8
Do	November	143	12.6	6.3	40.6		59.5
Do	December	1,421	1.1	2.0	4.4	.1	7.6
West Indian medlar	June	475	5.3	6.7	.2	3.8	16.0
Do	July	174	.6	1.2		1.7	3.5
Do	August	478	.2		.2		.4
Do	September	78	1.3				1.3
Yellow-wood	June	153		5.2			5.2
Kamani	February	250		19.6			19.6
Do	March	94		57.4			57.4
Yellow oleander	January	7			14.3	57.1	71.4
Do	February	56	1.8	1.8	8.9	28.6	41.1
Do	March	17	5.9	5.9		41.2	53.0

[a] The June collection of coffee came from the Waianæ Mountains. *Opius humilis* was first established here, but recently *Diachasma tryoni* was liberated.

TABLE II.—*Percentage of larval parasitism of Ceratitis capitata in Hawaii in 1917*—Con.

Host fruit.	Month of collection.	Number of larvæ emerging during first 2–6 days.	Percentage of parasitism.				
			Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total.
	1917.						
Yellow oleander	July	199	.....	13.6	13.6	47.7	74.9
Do	August	1,679	.4	7.5	14.4	32.0	54.3
Do	September	294	1.7	2.0	8.5	25.8	38.0
Do	October	104	.....	.....	25.0	14.4	39.4
Carambola	December	59	8.5	5.1	.....	6.8	20.4
Chinese orange	February	68	4.4	5.9	.....	1.5	11.8
Do	March	188	6.4	.5	1.6	1.1	9.6
Do	April	210	6.4	14.6	.5	.9	22.4
Do	May	100	17.0	11.0	.....	.....	28.0
Do	June	278	13.3	8.3	.....	2.9	24.5
Do	December	167	1.2	9.6	3.0	4.2	18.0
Ponay	June	129	.8	1.6	.....	.....	2.4
Do	July	386	.....	1.3	.2	2.2	3.7
Do	August	852	.1	26.3	.....	8.0	34.4
Guava	January	120	1.7	.8	.....	.....	2.5
Do	February	89	3.4	.....	.....	.....	3.4
Do	March	100	1.0	.....	.....	3.0	4.0
Do	April	472	2.1	.2	.4	.2	2.9
Do	May	91	8.8	.....	.....	.....	8.8
Do	July	83	6.0	3.6	.....	48.2	57.8
Do	August	446	1.8	46.9	.2	1.3	50.2
Do	November	153	.7	.....	.....	.....	.7
Loquat	January	19	10.5	21.1	31.6	.....	63.2
Do	February	109	1.8	11.9	62.4	5.5	81.6
Do	March	441	7.3	6.8	73.5	5.7	93.3
Do	December	41	4.9	4.9	51.2	22.0	83.0
Kona orange	January	.....	.....	.....	.....	.....	.....
Do	February	14	.....	7.1	.....	.....	7.1
Do	March	39	5.1	.....	2.6	.....	7.7
Do	May	14	7.1	14.2	7.1	7.1	35.5
Do	November	64	.....	.....	10.9	51.6	62.5
Mandarin orange	November	67	3.0	13.4	.....	.....	16.4
Do	December	50	.....	12.0	.....	4.0	16.0
Sapodilla	February	10	30.0	.....	.....	.....	30.0
Do	March	43	16.3	.....	.....	.....	16.3
Do	April	42	42.9	.....	.....	.....	42.9
White sapote	May	396	9.1	1.0	.....	1.0	11.1

Thus, as seen in Table III, nearly one-half of all the Mediterranean fruit-fly larvæ developing during the year were destroyed and this is entirely the result of parasitic importations. This achievement, solely due to the efforts of the Territorial Board of Agriculture and Forestry, is worthy of unusual commendation. Insufficient emphasis perhaps has been placed during recent years upon the utility of these parasites. The constant extinction of at least 45 per cent of all worms developing in fruit, apart from the destruction of this pest through other agencies already present in the islands, notable among them being the ant *Pheidole megacephala* Fabricius, without question greatly decreases the infestation of such fruits as the orange (*Citrus sinensis*) and certain

varieties of mango and avocado not ordinarily susceptible to prohibitive infestation except under conditions permitting an unchecked multiplication of the fly. This point has come to light during 1917, and the statement seems justified that a 50 per cent reduction in the numbers of the fly brings little relief to its favored host fruits, but that those fruits classed as unfavored hosts show a marked improvement in the degree of infestation, and some may become almost wholly free from larvæ. The propagation of such fruits and the encouragement of the parasitic method of control would seem to be the most favorable method of contending with this pest in Hawaii.

TABLE III.—*Total parasitism, by month, of all larvæ of Ceratitis capitata collected in Hawaii during 1917*

Month.	Number of larvæ.	Percentage of parasitism.					
		Opius humilis.	*Diachasma tryoni.*	*Diachasma fullawayi.*	*Tetrastichus giffardianus.*	Total in 1917.	Total in 1916.
January	6,183	39.0	15.6	2.0	2.4	59.0	6.98
February	4,568	20.0	8.6	3.0	1.3	32.9	19.5
March	5,901	27.1	22.5	12.6	1.3	63.5	14.7
April	2,861	9.0	27.6	5.5	1.2	43.3	37.64
May	4,439	8.8	26.4	3.4	2.3	40.9	26.69
June	5,919	11.7	16.3	.3	7.8	36.1	27.81
July	4,125	3.9	26.6	.9	19.6	51.0	18.52
August	8,726	2.8	16.4	4.7	9.2	33.1	37.5
September	9,047	5.2	31.3	13.2	2.7	52.4	45.2
October	11,309	7.2	16.4	13.2	8.4	45.2	44.3
November	5,919	17.2	22.6	11.6	20.9	72.3	44.3
December	3,142	5.3	16.7	5.2	7.0	34.2	44.1
Average, 1917	72,139	12.7	20.3	7.3	7.2	47.5	......
Average, 1916	83,304	17.2	13.3	2.1	.6	......	33.2

This 47 per cent reduction in the abundance of the fruit fly in Hawaii serves another purpose well worthy of mention. It is an important help contributing toward reducing the chances of its introduction to the mainland.

As shown elsewhere by the writers, the parasitism by the braconid *Opius humilis* Silvestri has been found highest during the coolest months of the year. Again during 1917 this was observed. The parasitism by *O. humilis* exceeded that of all of the other parasites combined in the months of January and February and was greater than that of any of the others taken separately during March. During the remaining months the parasite *Diachasma tryoni* was more abundant than the *O. humilis*. It is in the cool months of January, February, and March that the other parasites are so retarded that the *O. humilis* is enabled to gain a considerable foothold.

The average parasitism in all fruits by *Opius humilis* in 1915 was well above that of all the other parasites combined; in 1916 the total parasitism by it was 17.2 per cent, and by the others combined was 16 per cent; while during 1917 the other parasites had so reduced the *O. humilis* that the total by it was only 12.7 per cent and by the others combined was 34.8 per cent. This reduction of the Opius is proceeding slowly, but the species is not expected to be entirely annihilated. The coffee collections in the Waianæ Mountains at the head of the Waianæ Valley during 1917 were interesting. This was the only point at which *O. humilis* had been established when the collections were made. In February it was found parasitizing 89.4 per cent of the larvæ in the coffee; and in June 77.5 per cent were parasitized by it.

The parasite *Tetrastichus giffardianus* Silvestri was more abundantly recovered from fruit collections in 1917 than in any preceding year since its liberation in the islands in 1914. It was recovered, however, only in material collected about Honolulu. Its ability to penetrate to the interior of soft fruits broken or containing holes enables it when numerous to parasitize large numbers of larvæ in such fruits as the mango, the orange, or the common guava. The total average parasitism in larvæ from all fruits during the year was materially increased through the work of *T. giffardianus* in such fruits.

The total parasitism by all species during 1917 was 14.3 per cent higher than in 1916. The average infestation of all fruits combined was, however, not strikingly different from that of 1916.

As shown in Table III, the parasitism by *Opius humilis* is 4.5 per cent less than in 1916, while that of *Diachasma tryoni*, *D. fullawayi* Silvestri, and *Tetrastichus giffardianus* was 7.0 per cent, 5.2 per cent, and 6.6 per cent greater, respectively.

CORN-ROOTROT AND WHEATSCAB

[PRELIMINARY PAPER]

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In the progress of the investigations of rots of the root, stalk, and ear of Indian corn (*Zea mays*) being conducted by one of the authors (Hoffer) certain things have developed which have such an important bearing on certain phases of the wheatscab problem which is being investigated by the other authors that it has seemed desirable to publish a preliminary statement.

Field observations have shown a conspicuously greater abundance of wheatscab in fields where the wheat (*Triticum aestivum*) was grown immediately following corn that was infected with the Fusarium-rot of the root and stalk. This was especially true in Shelby County, Indiana, where wheat, according to a common practice, was sown in standing corn.

A similar condition was also noted in Dane County, Wisconsin, this summer, where spring wheat was grown immediately following a corn crop. Both in Indiana and in Wisconsin under these conditions abundant development of perithecia of *Gibberella* spp. was found on the old cornstalks remaining in these fields. These perithecia were mature and well filled with viable ascospores at the time when the wheat, in all cases observed, was in head.

Water suspensions of these ascospores both from Indiana and from Wisconsin cornstalks gave positive results when used as inocula on wheat heads. The inocula were applied by means of an atomizer spray. In some cases the heads were subsequently covered with glassine bags for three days, and in other cases no coverings were used. In all cases positive infections were obtained, while the controls sprayed with sterile water and similarly treated remained unaffected. The appearance of the infected heads thus artificially inoculated was identical with that of wheat heads naturally infected with scab.

Cultures from *Gibberella* spp. on old cornstalks have also proved virulently parasitic on the roots of corn plants grown both in large, sterile agar tubes and in sterilized pots of soil.

Similar results on both wheat and corn have been obtained by using cultures from naturally infected wheat heads.

The organisms from both sources have also been found to be similar morphologically. In view of the facts developed by this evidence, it seems certain that these are intercrop parasites which are of great impor-

tance in developing control measures for one of the rots of the root, stalk, and ear of corn and for scab of wheat. As both corn and wheat are such highly important food crops, it is imperative that the investigations bearing on any of these disease problems should be pushed forward with utmost vigor at the present time.

While the data are as yet somewhat fragmentary, it seems evident that, in order to lessen the losses from these diseases on corn and wheat, it is necessary to recognize this intercrop parasitism and develop field practices accordingly. In general, the use of the best-adapted, disease-free seed on clean soil should be practiced. The details of control measures for these diseases of corn and wheat are as yet not worked out, and no simple ones are evident. A crop rotation avoiding wheat following diseased corn is undoubtedly important, unless the cornstalks can be cut close to the ground, removed, and the remaining stubble plowed under before the wheat is planted. Badly scabbed wheat should not be used for seed. Ordinary seed treatments will not control wheatscab; hence, only clean seed on carefully prepared clean soil should be used.

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